FOOD TECHNOLOGY

Official Publication of
The Institute of Food Technologists
The Professional Society of Food Technologists

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Institute of Food Technologists

THE PROFESSIONAL SOCIETY OF FOOD TECHNOLOGISTS

The Institute of Food Technologists numbers among its active members leading scientists, technologists and engineers in the field of Food Technology.

The Institute invites your professional association with this select group of scientific and technological workers. Only those who are qualified by education, special training, or experience are extended this invitation.

Food Technology may be defined as the application of science and engineering to the production, processing, packaging, distribution, preparation and utilization of foods. Scientists, technologists and engineers engaged in Food Technology, executives, officials and supervisors under whose jurisdiction Food Technology operations are conducted, research investigators, teachers, and others working in the field of Food Technology, will find membership in the Institute worth many times its modest cost.

If you can meet the qualifications for membership, you owe it to yourself and to your business or profession to become a member.

OBJECTIVES

The Institute is a non-profit, professional, educational association to promote the application of science and engineering to the production, processing, packaging, distribution, preparation, and utilization of foods; it stimulates investigations into technological problems arising therein, it provides a medium for presenting and discussing the results of such investigations; it stimulates and provides means for the free interchange of technological information and ideas among food technologists; it promotes recognition of the role of food technologists in the technical direction of operations in these fields; all of these activities have the ultimate objective of providing better and adequate foods for mankind.

ORGANIZATION AND PROGRESS

Organized July 1, 1939, at Cambridge, Mass., with a membership of less than 100, the Institute has grown to more than 3,000 (1950). It is world-wide in scope, with members in North, Central, and South Americas, the Scandinavian countries, England, Holland, Germany, France, India, Australia, and New Zealand.

MEMBERSHIPS

Professional Members. Any person who is ethically qualified, and who has had training and experience in food technology, or any person who in the opinion of the Council is recognized as distinguished in the contributing sciences as they apply to foods, shall be eligible to be a Professional Member of the Institute. The minimum training which shall qualify a candidate for eligibility for Professional Member is, in general, graduation from a college, university, or similar institution in which he has majored in one or more of the sciences or branches of engineering associated with food technology. The minimum experience shall be three years experience in food technology. The Council may, at its discretion, waive any or all of the foregoing requirements in the case of a person who, thru long experience, has distinguished himself in the field of food technology.

Members. Any ethically qualified person, active in special or limited aspects of food technology, who is an administrator, director or executive under whose jurisdiction operations in food technology are conducted; or who is engaged in the dissemination of knowledge of food technology; or one whose work requires a general knowledge of the broad principles of food technology as it applies to the products, processes or equipment with which he or she is concerned; or one scientifically trained in and who has entered a career in food technology; shall be eligible to become a Member.

Student Members. Any ethically qualified person who has never been a Professional Member or Member of the Institute, who is registered as a student in an educational institution with at least Junior (third year) standing, who is a candidate for a Bachelor's or higher degree in one or more of the sciences or branches of engineering associated with food technology, shall be eligible for membership as a Student Member. This classification shall be his status only until the end of the calendar year in which he completes his schooling. When graduated and otherwise qualified as defined in the section on "Members," a Student Member may, upon application, be elected to Membership.

DUES

Professional Members and Members—$7.50 per year; Students—$2.50 per year. Dues include a subscription to FOOD TECHNOLOGY.

PUBLICATIONS

FOOD TECHNOLOGY, issued monthly, is the official journal of the Institute. It is published to advance the profession of food technology by disseminating the results of research and their practical application in industry. Contributors to its pages represent many fields of scientific and technological interest.

FOOD RESEARCH, published bi-monthly, is devoted to publication of the results of original investigations and occasional reviews in the fields of food and beverage technology, chemistry, and microbiology. FOOD RESEARCH is available to Institute members at the special rate of $4.50 per year.

REGIONAL SECTIONS

Where sufficient members (25 or more) live within commuting distance of a given point, a Regional Section may be established. Such Sections are able to hold meetings at more frequent intervals than the National group, thus affording the food technologists regular opportunities for an exchange of views and ideas with others in the area. There are presently 18 Regional Sections.

ANNUAL MEETINGS

Each year one of the regional sections acts as host to the Institute at an annual meeting. The program of this annual conference is designed to acquaint members with various phases of food production, processing and distribution, and to keep them abreast of current trends and technological developments. The speakers invited are those whose subjects will build a well-rounded program, of interest to the greatest number. Annual conferences are highlighted by exhibits of new equipment, processes and special ingredients.

AWARDS

The society administers the following awards:

NICHOLAS APPERT AWARD (Medal). Each year the Chicago Section of the Institute makes this award to a person whose contributions to the food industry make him distinguished and outstanding in the opinion of the national jury.

STEPHEN M. BABCOCK AWARD ($1,000.00). The purpose of this award, made by the Nutrition Foundation, is to encourage contributions to FOOD TECHNOLOGY which will have an influence on the nutritional well-being of the general population.

FOOD INDUSTRIES ACHIEVEMENT AWARD (Bronze Plaque). Presented bi-annually to the food company which has made the greatest achievement in food technology during the previous two years. The purpose of this award—presented by FOOD INDUSTRIES magazine—is to stimulate progress in the industrial application of food technology by food companies, and to encourage the cooperative teamwork between research and production that is the indispensable basis of industrial progress.

FUTURE ANNUAL CONFERENCES

1951 New York, N. Y.	1953 Boston, Mass.
1952 Grand Rapids, Mich.	1954 Los Angeles, Calif.

E. Engin
Ulrich

Food Technology

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FOOD TECHNOLOGY

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Volume IV, Number 1

January, 1950

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HOW TO BUY FOOD EQUIPMENT

When you are ready to consider food equipment of the type illustrated, here is a guide that will help you in selecting the best buys quickly:

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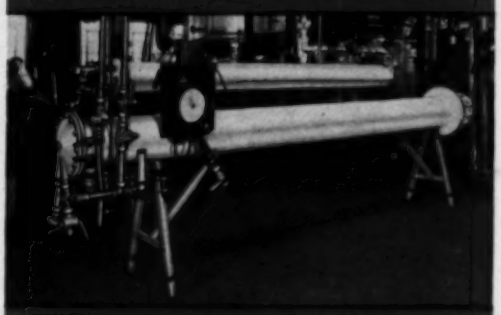
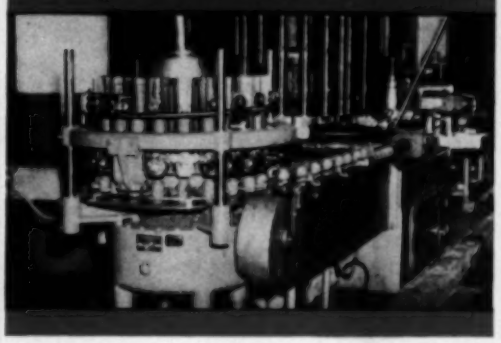


Heat exchangers for cooling and for heating liquids.



Filling machines for filling hot or cold liquids; semi-liquids and semi-solids in cans, jars and other containers.

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Help for Overseas Research

It is no longer necessary to tell members of IFT that libraries in Europe suffered disastrously during the war, as did also facilities for conducting technical and scientific research and means of supporting research. That fact has been told to us many times.

We can make a valuable contribution to science and technology by sending literature to the research workers of Europe. Anything we can do along this line will reduce the handicaps under which those research workers now labor.

We are informed that the facilities of CARE are available for sending technical and professional information to overseas libraries, universities and research groups. Mr. Milton L. Smith, Director of the Educational Unit of CARE states that our professional overseas counterparts are very appreciative of any contributions.

The Executive Committee of the American Association for the Advancement of Science proposed that its associated members be invited to cooperate and Dr. Howard A. Meyerhoff, Administrative Secretary of AAAS, has written the Institute of Food Technologists as follows:

> "Many of us, both as individuals and as members of professional groups, have made contributions to the physical rehabilitation of our fellow scientists in war-devastated countries. Now that the urgent need for keeping our friends alive has somewhat abated, it seems to me that we should turn our minds towards restoring in part the losses sustained in the realm of the intellect.
>
> "The full losses which the universities and libraries of Europe and Asia suffered during the war will probably never be known, but all of us are aware of the great lag in scientific progress overseas, due to the destruction or confiscation of literature, equipment and even men.
>
> "Our commanding position today in scientific fields is largely due to knowledge acquired over the centuries in Europe and transmitted to us in the past from institutions abroad. Consequently it may be said that the program worked out by CARE, in collaboration with UNESCO, under which contributions will be accepted for the sending of scientific and professional books to the libraries overseas, presents us with an opportunity to repay some of the debt we owe to the wells of learning from which we have drunk so deeply.
>
> "I believe that the facts about this program should be placed in the hands of every scientist in this country, and I urge the support of your organization in a cause which I am certain will aid the advancement of science—both abroad and here."

IFT has formulated no specific plan for participating as an organization in this worthy cause but Colonel C. S. Lawrence, Executive Secretary, 222 West Adams St., Chicago, Illinois, will be glad to hear from anyone who wishes to make a contribution in the name of the Institute. Personal contributions may be made directly through CARE, 50 Broad Street, New York 4, N. Y.

Book Review

PRACTICAL DAIRY BACTERIOLOGY, by Paul R. Elliker, McGraw-Hill Book Co. Inc., New York, 1949, 391 pages. Price $4.00.

The fundamental principles of microbiology are clearly explained in terms that may be easily understood by dairy students, farmers, inspectors, field service men and plant workers for whom the book is intended. Explanations of the feeding habits of microorganisms and the effect of their growth on the medium in which they live give a basis for understanding the processes involved in the manufacture of cultured milks and cheese which are discussed rather fully. Description of the processes of manufacturing condensed, evaporated, powdered milks and ice cream also are included along with measures for sanitary control during processing.

Methods of destroying bacteria and inhibiting their growth are discussed as are the practical applications of such methods in the sanitary control of milk supplies and of milk and milk products processing plants. Laboratory tests for determining the sanitary quality of milk, cream and milk products are discussed in sufficient detail to give a basic understanding of their application and interpretation. A chapter is devoted to cleaning dairy equipment including a discussion of the principles of cleansing and germicidal treatment and their practical application to various types of equipment.

It is a good book especially for short-course and in-service training of field and plant personnel in the basic principles of bacteriology and their practical application in the broad milk field.

WALTER D. TIEDEMAN.

FREEZE-DRYING (Drying by Sublimation), by Earl W. Flosdorf, Reinhold Publishing Corporation, New York, N. Y., 1949, 280 pages. Price $5.00.

This is not a large book but it is comprehensive, clear and concise. The author is well-qualified by pioneering experience in many phases of freeze-drying and by an exceptional clarity of expression, to reveal developments in freeze-drying during the past fifteen years in relation to medical, food and industrial products and also to plastic-imbedding of biological exhibits.

Basic principles are outlined; applications and equipment are described; and changes in products during drying and storage are discussed. Problems and prospects are indicated. Patent lists and bibliographies appear reasonably complete. Tables, charts and illustrations are abundant and excellent. The index is good.

The book has much to recommend it as a reference and text for all who are interested in technological aspects of the freeze-drying of biological products. Of

special interest to food technologists will be sections describing equipment, methods, and application of freeze-drying to fruits, meats, sea foods, milk, vegetables and other foods. Briefly summarized, freeze-drying provides a means for preserving many foods in a form which (a) is easy to reconstitute, (b) is greatly reduced in weight but not in bulk, and (c) if dried to less than one-half percent of moisture and sealed in gas-tight containers, freeze-dried foods may be stored and transported at temperature extremes with less change in flavor and food value than any other method for handling perishable foods.

The relatively higher cost of freeze-drying of foods can be justified where special problems are encountered such as in military applications, and in expeditions operating in locations where food-supply presents difficulties. As improvements occur in large scale equipment and as production volume increases, costs will be lowered and competitive areas of marketing will be extended.

Because it covers applications in widely separated fields, the usefulness of the book as a reference would be augmented if the author included a glossary of technical terms. There the reader could quickly locate the definition of any term with which he was not familiar. Such a glossary might be prepared and pasted in the present edition.

In future editions, the detailed explanation of the McLeod Gage beginning on page 54 might advantageously be accompanied by a series of drawings graphically presenting the features of this important instrument.

A section describing and comparing methods for determining the moisture content of dried products would also enhance the value of the book as a complete reference source in its field.

In its present form, the book is easily the most important contribution to date to the literature of Freeze-Drying.

J. L. Heid.

FINANCIAL STATEMENT

THE INSTITUTE OF FOOD TECHNOLOGISTS

at the close of business December 30, 1949

ASSETS

Cash on deposit with First National Bank, Chicago, Illinois:		
Operating Fund (subject to withdrawal on signature of Executive Secretary)	$ 1,253.16	
General Fund (subject to withdrawal only on signatures of both Treasurer and Executive Secretary)	19,673.07	
Total		$20,926.23
Accounts Receivable (subscriptions to Food Technology, advertising, sales of back numbers)		729.02
Publications (back numbers of Proceedings and Food Technology carried at cost. This is an item of doubtful value, as much of this stock will eventually have to be sold as waste paper)		5,682.90
Furniture and Fixtures (office equipment carried at cost or estimated value and depreciated 20% per year)		380.00
Total		$27,718.15

LIABILITIES

Accounts Payable (Income Tax withheld, and Social Security Taxes of employer)	$ 76.80
Net Worth of The Institute	27,641.35
Total	$27,718.15

There is in the hands of the former Secretary a U. S. Savings Bond with a maturity value of $1,000.00. This is not included in the above statement as it has not been received.

OPERATING STATEMENT

October 1, 1949 to December 30, 1949

RECEIPTS

By transfer from predecessor	$16,748.78
Food Technology, advertising and subscription income ($3.75 of each member's dues is credited to subscriptions, in accordance with postal regulations)	8,930.89
Members dues	$ 4,888.00
Sale of back numbers of Food Technology	20.10
Total	$30,587.77

EXPENDITURES AND CHARGES INCURRED

Food Technology, printing, mailing and commissions on advertising		$ 5,097.39
Refund of overpayment of dues by members		29.38
Purchase of back numbers of Food Technology		175.50
Operating Expense, office rent, stationery, clerical hire, blank forms, freight charges on transfer of Secretary's office		2,026.25
President's Office, travel and clerical hire during transfer period		344.67
Editor's Office, postage and incidentals, (the principal item of Editor's expense is paid in advance twice yearly. The next advance of $1,275.00 will not be due until after January 1st)		243.23
Furniture and Fixtures, purchase of adding machine		40.00
Secretary's salary and travel:		
Salary and Social Security Tax	$ 1,212.00	
Travel	82.73	
Total		1,294.73
Advances to Committee for 1950 Annual Meeting		342.19
Printing 5,000 copies of descriptive folders of the Institute for use of Membership Committees		145.00
Total		$ 9,738.34
Expenditures	$ 9,661.54	
Balance remaining in Accounts Payable	76.80	
Total		$ 9,738.34

Charles S. Lawrence
Executive Secretary

Effect of Storage Temperature on Quality of Frozen Peas[a]

F. E. LINDQUIST, W. C. DIETRICH, AND MILDRED M. BOGGS

Western Regional Research Laboratory,[b] Albany 6, California

Carefully prepared samples of frozen peas were stored at —10°, 0°, and 10° F. The stored material was examined at intervals by a panel of experienced judges to determine the rate at which changes in quality occurred. Appraisal was made by the triangular plan and results were analyzed for significance by the Chi-square method. Detailed data are presented and discussed.

The present study was undertaken to investigate the effect of temperature of storage on the quality of frozen peas and at the same time to determine, as far as possible, the rate at which such changes occur.

As food freezing has developed, there has been a change in recommended storage temperatures for various frozen products. In a recent paper, Woodroof and Shelor *(22)* point out that prior to 1930 temperatures from 10° to 20° F. were considered satisfactory by many for the storage of frozen foods. These authors also suggest that for some products with a quick turnover, 10° to 15° F. may be satisfactory, but that when the material is to be held for a long period —10° to —20° F. should be used. Morris and Barker *(16)* report that —20° F. is preferable to —10° F. for frozen peas. Lowe *(13)* states that peas stored at —10° F. were of better quality than those stored at 0° F. and of much better quality than those stored at 10° F. Powers *(20)* recommends that storage rooms for frozen vegetables should be kept at —10° F. Gortner et al. *(6)* state that it is important to store frozen foods at 0° F. or lower, if eating qualities and nutritional values are to be maintained from one season to the next. Thus, it is apparent that the trend in research on the storage of frozen foods has been toward recommendation of lower storage temperatures.

Procedures

Five lots of peas were used in the preparation of the samples. The variety was Ferry-Morse No. 9, a Laxton's Progress type. The raw material was obtained over an interval of nearly 3 weeks from fields being harvested for fresh market. The pods were iced within a few minutes after harvest and transported from field to laboratory where they were placed in storage at 33° F. overnight. Eighty pounds of finely-crushed ice was used for each hundred pounds of peas. Processing was carried out the following morning.

Earlier studies by Campbell and Diehl *(4)* and more recent studies by Boggs and Talburt *(2)* of this Laboratory indicated that peas in the pod, when stored at 33° F., maintain good quality for periods longer than those used for storage of raw material in this study. Hence, the raw material suffered no marked deterioration in quality.

The peas were podded and graded mechanically. Only peas of sieve sizes 3 to 5 inclusive ($^{11}/_{32}$ to $^{13}/_{32}$ inch in diameter) were used. The graded material (tenderometer readings from 76 to 81 for the five lots) was placed on stainless steel trays at a loading rate of 1.5 pounds per square foot, washed and then scalded in a draper-type, belt steam blancher at 212° F. for 70 seconds. Adequacy of blanching time was determined by the peroxidase test developed by Masure and Campbell *(15)*. After scalding, the peas were cooled immediately by a fog spray drawn through the loaded trays with the aid of a suction fan. Cooling continued for 2 minutes. The peas were then drained to remove excess water and frozen in an air blast at —10° F. The frozen material was packaged in No. 2 plain tin cans, hermetically-sealed.

Samples were stored at —10° F. until all five lots were processed. At this time, parts of each lot were placed in storage at 10° and 0° F., while the remainder was kept at —10° F. The samples were moved to each of the higher temperatures in rotation. The scheme followed was such that on each day of one work week, samples from one lot were placed in storage at 0° F. and on each day of the following week samples of each of the lots were placed in storage at 10° F. in the same order.

The samples were removed from storage at intervals and were examined by a group of experienced judges for differences in flavor, color, or texture. The methods used are discussed in the section on Appraisal of Samples.

Campbell *(3)* suggested that the loss of green color in peas stored at insufficiently low temperatures is probably due to conversion of chlorophyll to pheophytin. In view of this suggestion, measurements of chlorophyll conversion were made at each examination period. At the same time, measurements of color reflectance were made and ascorbic acid content was determined.

The measurement of conversion of chlorophyll to pheophytin has been described by Dutton, Bailey and Kohake *(5)*. The final determination was made on an acetone solution of pigments according to the spectrophotometric method of Mackinney and Weast *(14)*.

The measurements of reflectance color were made with a recording spectrophotometer *(7)* and a photoelectric reflectometer *(10)*. Samples were taken at each appraisal period and determinations were made on cooked peas, both whole and homogenized. A trichromatic description of the color was obtained from the spectrophotometric results, by standard methods *(11)*. Luminous reflectance and yellowness were calculated from the reflectometer data *(8)*. Ascorbic acid was determined by the method of Loeffler and Ponting *(12)* as modified by Ponting *(19)*.

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[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, California, July 13, 1949.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

At intervals of approximately 16 weeks, the following determinations were made on the samples: total solids, alcohol-insoluble solids, total sugars, total nitrogen, and pH. In all cases, except for determination of pH, the samples were made up in the form of alcohol slurries in which a known weight of peas was thoroughly blended with sufficient alcohol to give a final alcohol concentration of 80 percent.

Total solids were determined on weighed samples of slurry, dried to apparent dryness on a steam bath, followed by drying in an air oven at 70° C. overnight and subsequent drying in a vacuum oven (about 29 inches of mercury) at 70° C. for 48 hours. Alcohol-insoluble solids and total sugars were determined by a slight modification of the method described by Moyer and Holgate *(17)* in which the alcohol-washed solids were dried in the manner described above. The alcohol washings which contained the sugars were deproteinated with neutral lead acetate, and excess of lead removed with disodium phosphate. After proper volume adjustments and removal of lead precipitate, aliquots of the solutions were hydrolyzed for 10 minutes at 100° C. in the presence of hydrochloric acid at a final concentration of about ⅔ N. The hydrolyzates were further diluted and aliquots, after neutralization, were analyzed for sugar content with the copper-reagent and iodometric procedure described by Somogyi *(21a, b)*. Total nitrogen was determined by the Kjeldahl-Gunning-Arnold method, and crude protein was estimated by multiplying these values by the factor 6.25. pH was determined on slurries made by blending equal weights of peas and distilled water.

Results

The lines of regression for the conversion of chlorophyll to pheophytin and for the ascorbic acid content of the samples on storage time are shown in Figures 1 and 2, respectively. The standard error of estimate is indicated in each case by a vertical line. These data, together with the correlation coefficients, are given in Table 1a and b.

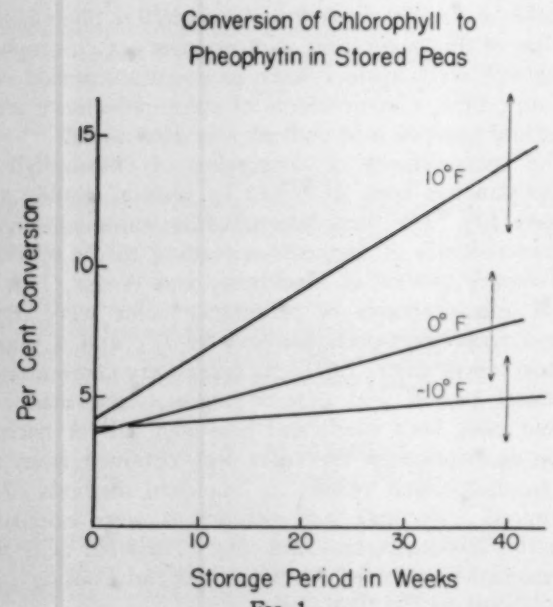

FIG. 1.

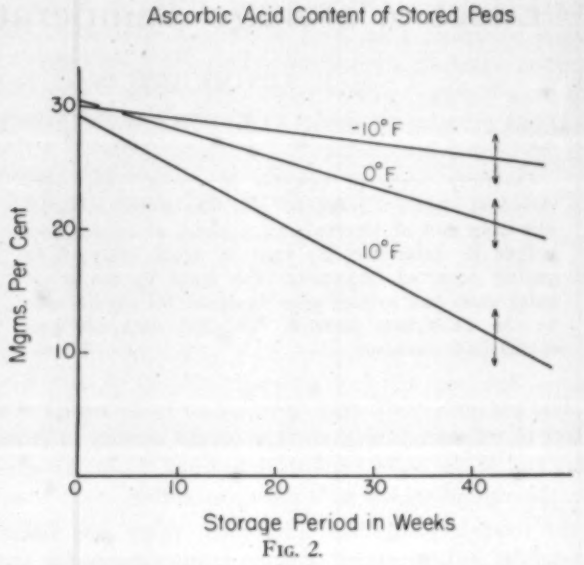

FIG. 2.

TABLE 1a

Chlorophyll Conversion in Stored Peas

Storage Temperature (° F.)	Correlation Coefficient	Slope	Standard Error of Estimate
—10	0.22	0.029	1.60
0	0.56	0.106	2.05
10	0.83	0.338	2.68

TABLE 1b

Ascorbic Acid Content of Stored Peas

Storage Temperature (° F.)	Correlation Coefficient	Slope	Standard Error of Estimate
—10	—0.58	—0.104	1.99
0	—0.87	—0.238	1.82
10	—0.91	—0.422	2.13

In the calculations, the values from all five lots of peas were combined and treated as one single large set of data. This fact accounts at least in part for the large values of the standard error of estimate, since the magnitude of the changes from lot to lot were quite different. However, the trends were the same in each case, whether the lots were considered individually or as a group.

The data for the rate of conversion of chlorophyll to pheophytin at 10° F. and for the rate of change of ascorbic acid content at 0° and 10° F. show a high degree of correlation with storage time, while correlations for the other data are not as high. In general, however, trends are clearly apparent, and the averages (Figures 1 and 2) point to the fact that as temperature of storage is increased, rate of conversion of chlorophyll to pheophytin and loss of ascorbic acid are increased.

The measurements of reflectance color were not as clearly defined as were measurements of conversion of chlorophyll to pheophytin. The results, in general, showed that as temperature increased there was a gradual decrease in brightness (Munsell value) and in purity (Munsell chroma) and an increase in dominant wave length (Munsell hue). The latter indicates that the sample was increasing in yellowness. This is un-

doubtedly associated with the conversion of chlorophyll to pheophytin.

With the exception of changes in ascorbic acid content of stored peas noted above, no significant changes in chemical composition were observed in any of the samples. Hence, analytical data are not presented. It suffices to say that the total solids content, alcohol-insoluble solids content, content of total sugars, and pH remained essentially constant for each of the lots throughout the entire period of storage.

The content of total solids in the five lots of raw material varied from 20.1 to 21.4%. In the processed samples, total solids ranged from 19.4 to 21.0% and total sugars from 6.1 to 7.1%.

Appraisal of Samples

The procedure used for cooking the samples to be appraised by the judges was to enter 300 grams of solidly frozen peas into half their weight of boiling water, which had been boiled five minutes to remove dissolved carbon dioxide. After the water had again returned to a boil, the containers were covered and cooking proceeded for five minutes. The water was then drained from the samples, which were presented to the judges for appraisal.

The presence or absence of differences between samples was determined by 10 to 16 trained, selected judges. The triangular test desccribed by Helm and Trolle *(9)* was used. At each judging period, duplicate samples from a lot stored at a given temperature, for example 10° F., were compared with a single sample from the same lot but stored at another temperature, for example —10° F. The judges were asked whether there was any difference between the three samples and, if there was a difference, to check the duplicate samples. The data so obtained were analyzed by the Chi-square method to determine whether the number of judges who identified the duplicate samples departed significantly from the one-third expected by chance in a large number of trials.

One third of the number of judgments marked "no difference" was added to the number who identified the duplicate samples correctly, while the remaining two thirds was added to the number who checked the wrong samples as duplicates. These adjusted values for the combined results of the five replicate processing lots were analyzed.

In addition to the information usually obtained by the triangular test, the judges who thought there was a difference between the samples were asked to check the sample or samples which they considered best in flavor, texture, and color. Only the results of those judges who identified the duplicate samples were used in the Chi-square analysis of these data. Thus, the results are those of the most acute judges on each day. The number of judges expected to prefer each of two different samples is one-half rather than one-third, as in the triangular test. The procedure used with the "no difference" judgments in the triangular test, was followed here except that one-half of the "no difference" judgments, rather than one-third, was added to the number who preferred each sample.

The results of the appraisal are summarized in Table 2. The data for the comparison of samples stored at —10° and 10° F. clearly show that the entire panel detected a difference between the samples after 8 weeks of storage and after all longer periods. Flavor deterioration of the samples stored at 10° F. enabled the judges to identify duplicate samples. The most acute judges detected flavor deterioration after only 4 weeks. There was a trend showing better color and texture in the samples stored at —10° F., beginning at the twelfth week, but the color difference was not statistically significant until the twenty-fourth week and texture difference not until the thirty-second week.

None of the judging methods now in use give satisfactory measurements of differences between samples when considerable time elapses between evaluation periods. Some information regarding rate of quality change may be obtained, however, from percentage identification of duplicates and percentage preference for —10° F. samples.

TABLE 2

Judges' Results

Samples	Identification Data					Flavor		Texture		Color	
	Storage period	Total judgments	Identified duplicates	No difference	Chi-square*	Sample** preference	Chi-square*	Sample** preference	Chi-square*	Sample** preference	Chi-square*
	Weeks										
—10°, 10° F.	4	49	17	10	1.1	12-2	4.8	5-4	0.0	0-3	0.2
	8	45	25	6	13.2	21-2	13.0	6-2	0.4	4-5	0.0
	12	75	48	7	37.0	37-2	24.1	10-0	1.7	15-1	3.5
	16	69	54	2	63.4	51-3	40.9	15-3	2.2	15-1	3.1
	24	65	50	0	53.6	45-3	33.6	15-1	3.4	22-1	8.0
	32	71	61	0	86.0	58-1	51.4	31-2	12.9	45-0	31.7
—10°, 0° F.	4	51	21	14	5.9	14-7	1.7	3-6	0.2	6-2	0.4
	8	52	12	12	0.1	8-1	3.0	4-3	0.0	5-1	0.8
	12	75	30	17	6.2	19-5	5.6	5-4	0.0	2-6	0.3
	18	68	39	6	21.0	36-2	27.9	8-2	0.6	9-3	0.6
	20	68	34	5	10.3	24-10	5.0	9-6	0.1	5-2	0.1
	24	61	26	5	3.5	24-1	18.6	8-1	1.4	3-0	0.2
	28	68	22	8	0.2	18-3	8.9	2-1	0.0	4-0	0.4
	32	76	42	4	18.1	37-4	24.4	15-0	4.7	9-0	1.5
	36	68	40	0	18.7	35-4	22.5	20-3	6.4	7-3	0.2
	40	60	36	0	18.0	30-4	17.4	14-4	2.2	10-1	1.8
0°, 10° F.	43	68	56	0	71.3	51-3	39.4	30-2	13.0	49-0	41.1

*Chi-square of 3.8 is significant at 5% level, 6.6 at 1% level.

**The first number is the number of judges who preferred the sample stored at the lower temperature and the second number is the number of judges who preferred the samples stored at the higher temperature.

The percentage identification of duplicates by the entire panel in the 10° F. series, 35, 56, 64, 78, 77 and 86 for 4, 8, 12, 16, 24 and 32 weeks, respectively, show a trend for increasing difference in quality with increasing storage time. The percentage preference for —10° F. samples at the storage period listed above, 71, 84, 77, 94, 90, and 95, do not show the trend so clearly, because a large proportion of these more acute judges preferred —10° F. samples even at the early storage periods. It is of interest to note that the same persons were not the most acute judges on different days.

The results for the samples stored at —10° and 0° F. were not entirely consistent. Although the most acute judges detected a flavor deterioration at 12 weeks and after longer periods, there were several inconsistencies in the identification data. The Chi-square values were significant at the five percent level at 4, 12, 18, 20, 32, 36 and 40 weeks of storage but were not significant at 8, 24, and 28 weeks. The data of individual judges have been examined carefully for an explanation of the irregularities observed after 8, 24, and 28 weeks. No obvious explanation was found. The decreased percentage of identification was characteristic of 11 of the 14 judges on all five days for each test. Thus, the disturbing inconsistencies must be attributed to factors beyond our present ability of control.

The rate of deterioration in this series is not clear. Percentage preference by the most acute judges for —10° F. samples, 67, 67, 63, 92, 71, 92, 82, 88, 88, and 83 for 4, 8, 12, 18, 20, 24, 28, 32, 36, and 40 weeks, respectively, indicate that the difference between samples was fairly constant between 4 and 12 weeks, then increased somewhat in the next 4 months. The identification data for the entire panel, 41, 23, 40, 57, 50, 43, 32, 55, 59, 60 percent show no systematic pattern of change. The most plausible explanation is that differences between —10° and 0° F. samples were on the margin of threshold of judges even when the peas were stored for 40 weeks.

Color and texture changed slowly, if at all, in the samples stored at 0° F. The difference in color between the samples stored at —10° F. and those at 0° F. was not significant at any period of storage up to 40 weeks. There was a trend showing some deterioration of texture in the samples stored at 0° F., beginning at the thirty-second week, but the deterioration at this storage temperature was primarily one of flavor.

As a supplement to the main study, a comparison of the samples stored at 0° and 10° F. was made at one storage period only: 43 weeks. All of the data clearly show that the samples stored at 10° F. were inferior in flavor, texture and color.

In the hope of obtaining a comparison of the material used in the present study with material marketed commercially, particularly with regard to flavor, the samples stored at the various temperatures for 46 to 47 weeks were compared with samples packed by four of the larger packers of frozen food. To be assured that the samples used were as uniform as possible, a case of each of the four commercial samples was obtained directly from the warehouse of the wholesale distributor. It was soon apparent that a comparison of the commercial and experimental samples would be difficult because of the marked difference between the two, both with regard to flavor and maturity. The experimental samples had been prepared from young, tender peas processed with every precaution to avoid any changes due to delay between shelling and blanching and blanching and freezing. Hence, any differences in flavor between the experimental samples were largely a result of differences in storage temperature. On the other hand, the flavor of the commercial samples could have been affected not only by storage temperature but also by delay between vining and blanching and by differences in maturity (alcohol insoluble solids of commercial samples varied from 10.9 to 16.3%, while those of experimental samples varied from 9.9 to 11.0%). Thus, it cannot be emphasized too strongly that when comparisons of the type made in this study are to be carried out, extreme care must be exercised in the control of the raw material used in the preparation of the samples.

Discussion

The data reported clearly show the effect of temperature of storage on the retention of quality of frozen peas.

From the standpoint of economics alone, it would be an advantage to both warehousemen and wholesale distributors of frozen foods to store these materials at as high a temperature as possible, because of the progressive increase in the cost per unit of refrigeration as the storage temperatures are lowered. Storage at 10° F. was obviously unsatisfactory, because of the changes in flavor which occurred very early in the storage period and also because of the fact that the material was stored near the upper limits of safety as far as microbial spoilage is concerned. It has been pointed out by Berry *(1)*, for instance, that the deadline for microbial growth is near 15° F.

It is apparent that it would be poor commercial practice to store frozen foods at temperatures even approaching the upper limits of safety just mentioned and that certain sacrifices must be made economically, in order to maintain maximum quality—a factor which is all important, particularly in frozen foods. The data presented in this study clearly indicate that at storage temperatures as low as 0° F., flavor deterioration occurs after a relatively short period of storage (12 weeks) if only the judgments of the most acute judges are considered. Hence, in the range of temperatures studied, —10° F. was the most desirable storage temperature for the maintenance of quality of frozen peas over long periods of storage. This is in agreement with work cited earlier. It would seem that the range of temperatures used represents a critical zone for frozen peas, evidenced by the marked loss in quality of the frozen material with increase in storage temperature. However, it would be of considerable interest to know whether storage at —20° F. would bring about a retention in quality comparable to that noted as the storage temperature was lowered from 0° to —10° F.

The changes in quality with storage temperature noted in this work are those for young, tender, carefully harvested peas. It is quite possible, if raw material at a more advanced stage of maturity had been

used, quite different rates of change with storage temperature might have been noted. It is hoped that this point can be investigated in the near future.

Summary

The effect of temperature of storage on the quality of frozen peas has been investigated. Five lots of peas of comparabale maturity were stored at —10°, 0° and 10° F. The samples from each lot were examined at intervals by the judges to determine any differences in flavor, texture, or color between the samples stored at —10° F. and those stored at the higher temperatures. The data were analyzed for significance by the Chi-square method.

The samples stored at 10° F., when compared with those stored at —10° F., showed a significant flavor deterioration at the fourth week of storage (based on the judgments of the most acute judges), which persisted throughout the entire period of examination. A significant difference in color and texture was noted at the 24-week and 32-week storage interval, respectively.

In a similar comparison between the samples stored at —10° and 0° F., the data of the most acute judges showed a deterioration in flavor in the sample stored at the higher temperatures, beginning at the 12-week interval and extending throughout the entire storage period. No significant differences were noted in color or texture up to 40 weeks of storage.

One comparison of the samples stored at 0° with those stored at 10° F. at the 43-week interval clearly showed that the samples stored at the higher temperature were very inferior in flavor, texture and color.

Physical measurements showed that the rate of conversion of chlorophyll to pheophytin was greatest in the samples stored at 10° F. and least in those stored at —10° F. The rate of loss of ascorbic acid followed the same trend. No other changes in chemical composition were noted.

Acknowledgment is gratefully given to Mr. Glen F. Bailey for carrying out the measurements on the conversion of chlorophyll to pheophytin, to Dr. E. J. Eastmond who carried out color reflectance measurements, to Mrs. Alice C. Ward for assistance in the appraisal of the samples, and to Miss Marion K. Lee for assistance in all of the chemical analyses of the samples.

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"Electro-Sensing—An Advanced Measuring Tool for Food Process Control"[a]

LLOYD E. SLATER

Minneapolis-Honeywell Regulator Co., Brown Instruments Division, Philadelphia, Pa.

Most changes in a product or its environment, while in process, can be gauged by a tiny variable electrical signal, generated through the action of recently developed sensing elements. This concept of "electro-sensing" has been made possible through the development of vacuum tube instrumentation which is now available for the critical measurement and control of such processing factors as pH, density, composition as well as temperature, pressure and flow.

Measurement is often called the foundation of all science. In the Food Industry, as indeed in almost every other industry, the quality and yield of final product is closely related to the techniques of measuring, and thereby controlling the various processing factors. Advanced methods of measurement are today eliminating many of the "rule-of-thumb" procedures which have characterized food manufacture. The old fashioned artisan or craftsman is disappearing from the food plant, being replaced by the technician who is capable of using modern instrumentation in scientific production.

The object of this paper is to briefly survey some recent developments in the field of measurement. The application of new measuring tools to food processing depends primarily upon the food technologist. It is his imagination and experimentation which usually allows the benefits possible from such devices to materialize in his industry.

The most usable majority of recently developed measuring devices can be classified under one title—"Electro-sensing" elements. This title merely implies, of course, a means of sensing something electrically. Translated into the working vocabulary of instrumentation, an "Electro-sensing" element is one which provides a variable electrical signal as an index of measurement.

The most familiar, perhaps even the progenitor, of the "Electro-sensing" elements is the thermocouple, which generates an electrical millivoltage in proportion to its temperature change. Another "Electro-sensing" element, also quite familiar to the food technologist, is the glass pH electrode, which provides a tiny voltage proportional to the hydrogen ion content of a solution.

The chief virtue of "Electro-sensing" is that it enables us to measure smaller things. Minute changes in a product quality or process variable can now be detected, and with great accuracy. It is easy to understand this exceptional ability when it is realized that "Electro-sensing" elements provide the measuring and controlling instrument with the lightest and most mathematically concise measuring agents, electrons and photons.

The electrical power available from an "Electro-sensing" device is rarely of sufficient magnitude to directly operate a calibrated pointer or control element. This is especially true where measurement of a high sensitivity and accuracy is required. In such work, where very small increment changes in a process or product are to be detected and possibly controlled, correspondingly minute electrical quantities are involved. Obviously, in order to actuate a modern precision recording and controlling instrument, these signals must be amplified. A familiar parallel, one that is too close to be called an analogy, is the manner in which your home radio set amplifies the tiny signals received on its antenna to the point where they are capable of producing audible sounds on a loud speaker.

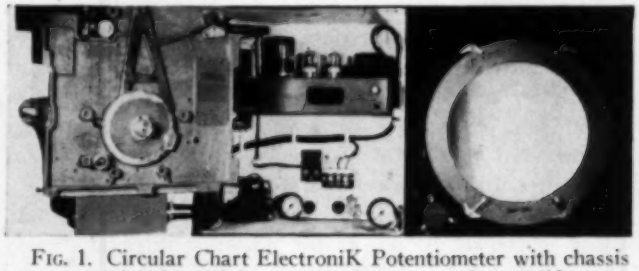

FIG. 1. Circular Chart ElectroniK Potentiometer with chassis swung out showing driving gear and vacuum tube amplifier.

Needless to say, the full capabilities of "Electro-sensing" elements were not brought into play until the advent of the electron tube as an amplifier in the measuring instrument. The use of this principle came surprisingly late in the instrument industry, as it did in most industries other than communications, the conception and development of an electronic recorder occurring less than ten years ago. However, since its initial inception, those few years ago, electronic instrumentation has advanced at a very dynamic pace and has been found to be capable of accommodating new and unusual types of measuring elements as soon as they are created.

An interesting development has appeared as electronics have been applied to instrumentation. It has been found that the same basic electronic instrument can be used with various "Electro-sensing" devices and thereby be employed to measure and control many diverse variables. All that is usually necessary, to adopt the basic electronic instrument to any one of these variables, is simply to calibrate it in terms of the variable and provide it with a suitable range.

This adaptability is easily understood when it is realized that all the receiving instrument does is operate on a voltage signal. Whether this signal is self-generated, as by a thermocouple or glass electrode, or due to a variation of current flow through a resistor, as in the resistance bulb or strain gauge, the same electronic means are used to amplify it to a usable value for indicating, recording and controlling.

[a] Presented at the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 11, 1949.

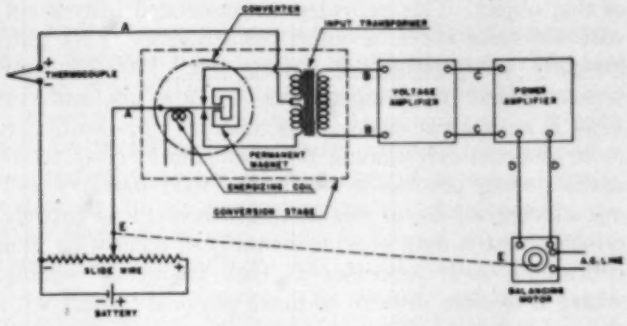

FIG. 2. Schematic diagram of Continuous Balance System.

A brief review of the "null-balance" electronic potentiometer will help to clarify this point. The measuring circuit of this instrument consists of a bridge of wire resistors with a constant D. C. voltage imposed upon it. This bridge is "in balance" when the sensing element, connected into the circuit, is static, or not reflecting a change. When an "unbalanced voltage" is received in the measuring circuit, due to a signal from the sensing element, this will be electrically magnified by the electron-tube amplifier in the instrument to a suitable power to operate a rebalancing motor. This motor then repositions a slide wire contactor operating on one of the resistors in the measuring circuit to thus bring the circuit back into "balance." The rebalancing motion of the motor also brings the recording pen and/or control mechanism into its new reading. An important agent in the success of this transaction is the convertor, which changes the D. C. signal into an A. C. voltage of proportional magnitude. It is this alternating current signal which is, of course, amplified and used to drive the compact, reversible two-phase balancing motor.

While actually simple in its operating fundamentals, the electronic instrument has received relatively sparse usage in the food industry. This has not been due to any less exacting demands in the food industry or to the inability of the electronic instrument to stand up to the rigours of most food plant conditions. The lack of widespread usage is more probably due to the average food plant engineer's unfamiliarity with the fundamental principles and possibilities of the electron tube. This device, because of its amazing accomplishments in the field of communications—its first user, has retained an aura of mystery for engineers in other industries. However, advanced methods of processing and higher quality standards have almost demanded the use of electronic principles in many places, and through this practical usage will occur a general increase in the ability of the food industry to apply this tool.

In actuality, despite this aura of mystery surrounding electronics, the continuous-balance potentiometer has an inherent sturdiness and reliability which is unmatched by equivalent mechanical instruments. It contains very few moving parts, and these, due to the powerful driving torque of the balancing motor, can be built exceptionally rugged to withstand higher speeds of operation and mechanical wear. This sturdiness and reliability have been proven in the success of this instrument in such diverse applications as the test of aircraft in flight and the control of temperatures on a drop forge.

The actual gains in the quality of measurement afforded by electronic instrumentation have been shown to be improved standards of accuracy, sensitivity and reproducibility of measurement. Of particular interest in this paper, is the flexibility of measurement provided by the electronic principle. With this increased quality of measurement coupled with the flexibility of applying the electronic instrument with various "Electro-sensing" elements, it is now possible to carry on many of the precise analytical measurements of the control laboratory directly in the process stream, and thus to automatically correct for unwanted deviations.

The concluding section of this paper will discuss some of the "Electro-sensing" elements and their application, or possible application to food process control. Since, in many cases more than one of these elements are available to measure the same effect, it will be expeditious to discuss them in relation to the variable they are called upon to measure.

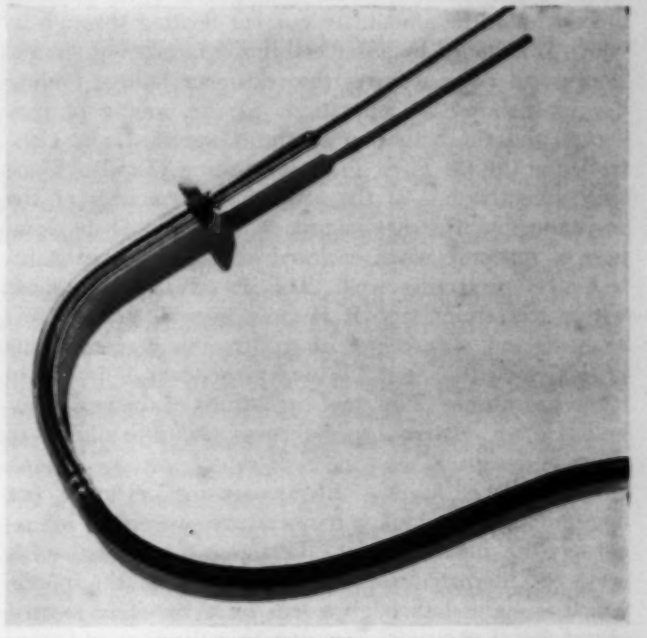

FIG. 3. Sanitary Thermocouple.

Temperature

The two best known and most frequently applied "Electro-sensing" elements for temperature measurement are the thermocouple and resistance bulb. The former, because of the amazing flexibility possible in its construction, has received greatest application in the food industry. It is widely used, for example, in heat-penetration studies of canned foods and food freezing studies. It is also used in the control of heat exchangers, such as flash pasteurizers and Votators. It is relatively inexpensive, very durable, and can be provided with a very high speed of measuring response.

The resistance bulb consists of a precisely wound coil of nickel or platinum wire on a form. Changes in temperature are reflected by changes in electrical resistance of this winding. This principle allows two

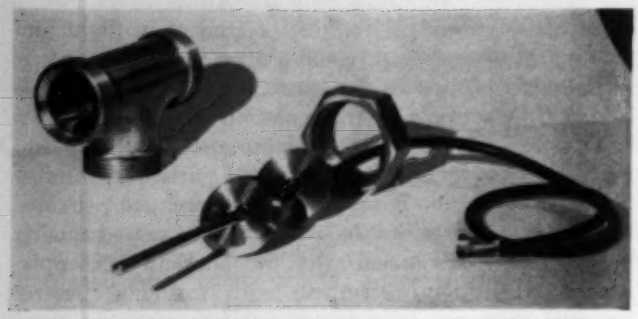

FIG. 4. Sanitary Resistance Bulb for Pipe Line Connection.

major advantages of the resistance bulb over the thermocouple; greater accuracy of measurement and the ability to be used with narrower temperature spans. The increased accuracy is due to the very precise resistance value which can be provided in a wound coil of high quality wire. Measuring spans as narrow as 20° F. are also possible with this element due to the fact there is not, as in the thermocouple, the limitation of a self-generated signal of tiny size. The magnitude of the signal received from a resistance bulb depends merely upon the amount of current flowing through it, which is supplied by a dry cell in the measuring circuit. Because of these features the resistance bulb is finding use in such precise operations as the boiling of preserves and the actuation of the Thermal Limit Controller in the H. T. S. T. pasteurization of milk. Until very recently, one of the serious shortcomings of the resistance bulb for use in process control was its slowness of response when enclosed in the necessary stainless steel protecting well. This factor has been dealt with successfully, and it is now possible to use this element and secure precise control in a continuous process to within $\pm 1/2°$ F. and as good as $\pm 1/8°$ F. in batch operations. The chief limitations of the resistance bulb are its relative expensiveness and inflexibility of physical design.

A fairly well known "Electro-sensing" element, but one quite new to the low temperature processing industries is the radiation unit. This element consists of a series of thermocouples in the form of a thermopile which is sighted through a lens on a remotely located object for the purpose of measuring the heat radiation of that object. This provides the associated instrument with a definite index of object temperatures. This unit, formerly only useful for objects over 1000° F., can now respond to temperatures as low as ambient (75° F.). A great deal of experimental work will have to be done in determining the heat emissivity of foodstuffs during processing before the food industry will see widespread use of this unit. However, it is entirely possible that it may provide a means of measuring and controlling such processes as high frequency heating where it is often difficult to make physical contact with the product for purpose of temperature measurement.

Another fairly new "Electro-sensing" element which may have excellent possibilities in the food industry is the Thermister. This is a resistance type unit composed of rare metal oxides which reflects a very large change in resistance upon temperature change—almost ten times that of copper. A thermister can be made as small as a pin head and have the form of beads, rods, disks, or flakes. Because of this tiny size it can, of course, be used to measure temperature in the most inaccessible places. An example is its current use to measure blood temperature by actual intravenous insertion in a living patient. Its large temperature resistance coefficient allows an extremely narrow temperature span in the associated instrument. In one commercial thermister application the control of a chemical solution is being held to within 1/20° F. with an instrument having a span of only 2° F. This unit will probably have considerable use in heat penetration studies in the food industry and also in precision laboratory and pilot plant measurements. Its chief limitation, at this point, is that each unit must be carefully calibrated with its associated instrument.

pH

While hydrogen ion concentration has been measured in the laboratory for many years, it is only very recently that industrial in-plant use of this principle has become possible. This has been due to the evolution of the glass electrode to the point where it is now feasible to insert this element into boiling aqueous solutions, highly alkaline solutions and under the usual severe operating conditions encountered industrially. The actual pH electrode assembly consists of three members: the glass electrode; a calomel reference electrode; and a resistance thermometer. The resistance thermometer

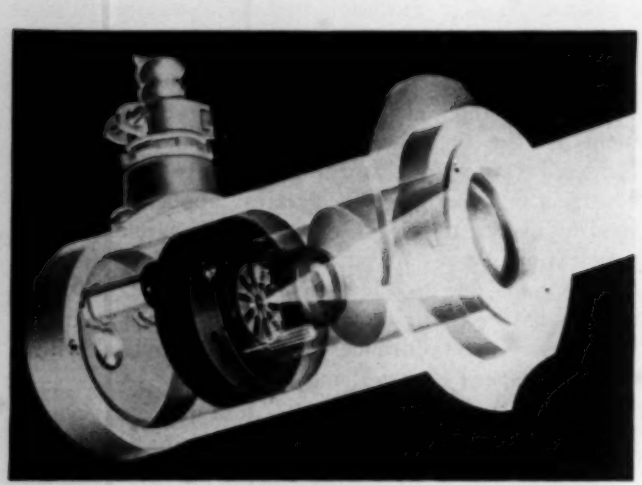

FIG. 5. Phantom view of Brown Radiation Unit.

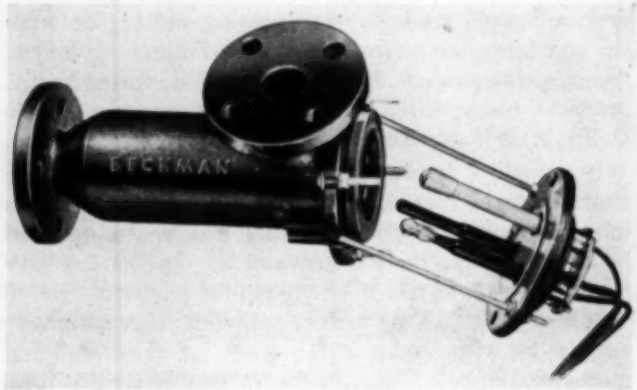

FIG. 6. Flow-type Beckman Glass Electrode.

automatically compensates for the effects of temperature upon the resistance of glass in the electrode. This assembly is available for immersion into a vessel or direct connection into a flow line. The pH electrode is used with a special high impedance vacuum tube amplifier before the electronic recorder-controller because of the extremely minute amount of current developed by the glass electrode in response to hydrogen ion concentration. The sugar industry was one of the first to use the glass electrode for commercial control, and most sugar mills now employ electronic pH control systems for the automatic continuous liming of raw juice. Other processes where this unit has proven feasible are the manufacture of casein, control of acidity in jelly making, and more recently the control of acidity in the ripening of cheese.

Conductivity

The conductivity cell provides a measurement of the electrical conductivity of a liquid. It consists of a pair of accurately spaced platinum electrodes within an insulating chamber which serves to isolate a portion of the liquid under test. As such, these electrodes effectively

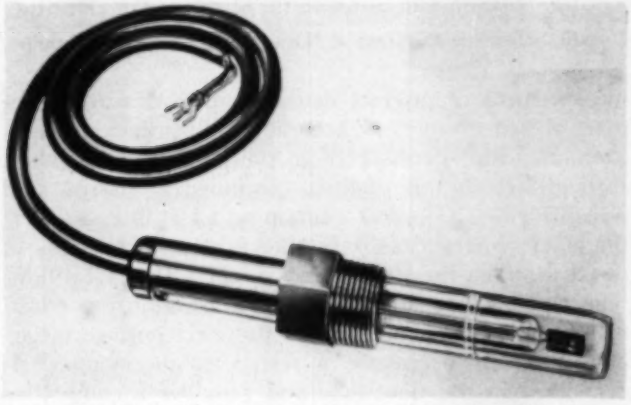

Fig. 7. Industrial Instruments Conductivity Cell.

use the solution being tested as an electrical resistor, the amount of current passed being a function of solution conductance. The cell is available as a dip or flow type assembly and in a variety of housing materials to meet the requirements of the process. It is exceedingly simple and rugged and has found use in controlling the concentration of lye peeling baths, brining solutions, etc. An interesting aspect of the possibilities of the conductivity cell is that this device can often be used to give an index of properties other than conductance itself. For example, the percentage of water in a liquid can often be gauged by the conductivity of that liquid. The actual composition analysis of material can often be reflected by conductivity measurement. An example of this is provided by the use of conductivity signaling controller to detect the addition or lack of addition of salt to a moving stream of tomato juice cocktail.

Pressure, Liquid Level and Flow

A war-born "Electro-sensing" device is currently being developed to allow the electronic measurement of direct pressures as well as liquid level and flow. This consists of a modified strain gauge, known as a Pressure

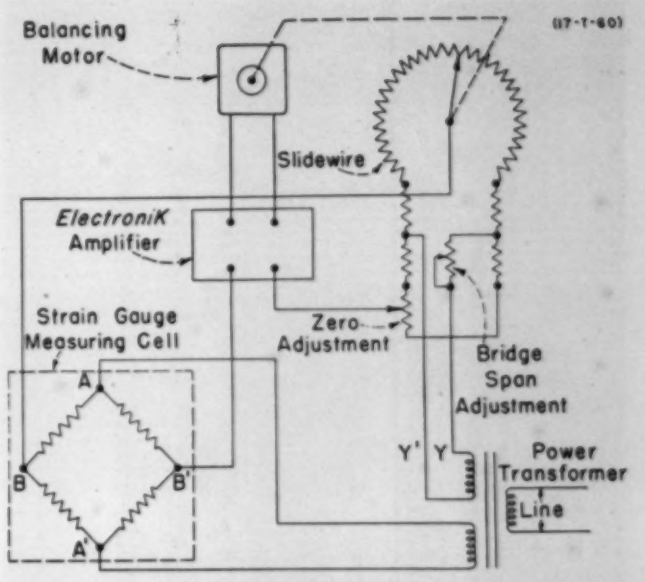

Fig. 8. Schematic Diagram of Strain Gauge Connection into measuring bridge of ElectroniK.

Pick-Up, which is a grid of fine alloy wires that undergo small linear changes in dimensions as a result of the pressure of the medium being contacted. This device makes use of the simple principle that when an electrical conductor is subjected to mechanical stress, its electrical resistance changes. It is connected up as one arm of the measuring bridge circuit of the electronic recorder-controller. A dual bridge of these devices, tapped in around an orifice in a pipe line, can be mounted together as an individual differential pressure pick-up to measure flow. A sanitary Pressure Pick-Up has already been developed and tried out with great promise of success in a number of food process applications. This unit has been designed for sanitary pipe line connection, is fully stainless steel housed, and contacts the foodstuff by means of a flexible diaphragm. Its initial use has been for the multiple measurement of liquid level in a number of storage tanks on a single electronic strip chart recorder. In this manner as many as sixteen remotely located electrical Pressure Pick-Ups can register liquid level on one centrally located chart. The chief advantages that this unit will provide over the available sanitary type pressure gauges and flowmeters are greater ruggedness, sensitivity and accuracy in the sensing element and, of course, the ability to utilize the electronic recorder-controller with its corresponding advantages. While still in its early stages of development and application, it is easy to foresee many possibilities for the sanitary Pressure Pick-Up in the food industry.

Humidity

An electrical humidity "pick-up" is now available that measures atmospheric moisture, and which can be used in conjunction with a basic electronic instrument for a very accurate control of this important variable. This sensing device, known as an Electric Hygrometer, consists of a small polystyrene insulating tube upon which is wound double spaced wiring of precious metal. The whole unit is, in turn, covered by a thin coating of a

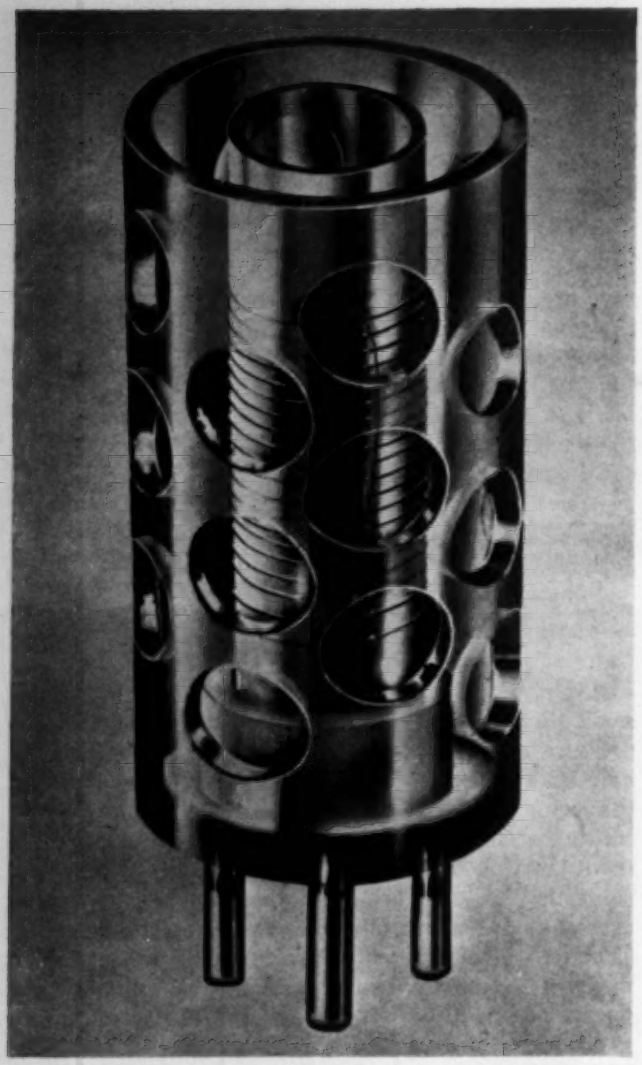

FIG. 9. Amico-Dunmore Humidity Sensing Element.

hygroscopic material, lithium chloride. This thin hygroscopic film loses or gains moisture and is in constant equilibrium with the surrounding atmosphere. Thus, the atmospheric humidity can be measured by measuring the electrical resistance of the element, since the electrical conductivity of the coating film is a function of its water content. To obtain high accuracy, eight of these elements are available to cover the range of from 7% to 100% relative humidity. The major advantages of this "Electro-sensing" device over its mechanical equivalent, the hair hygrometer, are exceedingly rapid response, very high sensitivity (changes as small as 0.5% are detected) and a guaranteed accuracy of $\pm 1.5\%$ R.H. An interesting application for this device, which is being worked out, is the determination of occluded moisture in grains and cereals. Other applications should appear in the critical control of food drying and packaging operations.

Density

The density or specific gravity of fluid food products is a major variable in most food processing, and one that usually requires a periodic laboratory check for control purposes. An "Electro-sensing" device, the "Densitrol," is now available for the continuous

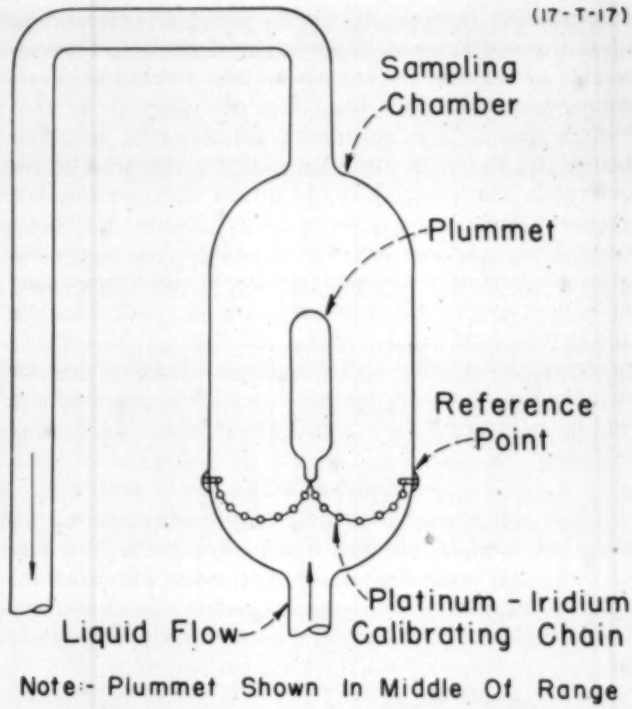

FIG. 10. Schematic Diagram of "Densitrol" Measuring Chamber.

measurement of product density with an amazing degree of sensitivity and accuracy. This device, in its sanitary form, consists of a stainless steel chamber, through which the product continuously passes, and which holds a plummet containing an iron core. The plummet rises and falls in this tube with changes in density of the fluid only, and is not affected by flow. The assembly is surrounded by a transmitting transformer which is connected to the electronic recorder-controller. If necessary, a resistance thermometer is immersed in the flow tube and provides a continuous compensation for temperature changes by feedback to the recorder input circuit, so that density is measured as though the process fluid were at a constant temperature. The sanitary "Densitrol" is currently being applied to the measurement and control of sugar syrup coming from an evaporator to within 1/10° Brix. It should have similar application in most other food condensing processes. Its major advantages are its ability to be simply connected into a process line for continuous measurement and its very excellent accuracy. The accuracy of the "Densitrol" increases with the narrowness of the measuring span, and on a span of from 1.0150 to 1.0250 Sp. G., for example, a guaranteed accuracy of .0003 Sp. G. is provided. Any calibration normally used in food processing, such as Baumé or Brix, is available. At present, the chief limitation of the "Densitrol" is that it is confined to the measurement of products with viscosities of 1000 centitpoise or less. This eliminates many viscous food products such as marshmallow and peanut butter.

The aforementioned "Electro-sensing" elements are just a few of a fast growing number of similar devices which are available, or in development, for the measurement of many other quantities. An especially interesting field of work is appearing in the "in-line" measure-

ment of actual product qualities. It is now possible to employ electronic means to evaluate such product qualities as colour, viscosity, moisture content, crystal structure and chemical composition.

In actual practice, however, accurate and instantaneous measurement has been found to be only a partial solution to the processors' problems. Careful study must be given to the factors causing the measured quality in the product and towards the means of controlling these conditioning variables. It is this field of flowing process stream control which presents the greatest challenge to the instrument engineer and industry technologist. As we today are amazed at the variety and scope of new measuring tools, so will our incredulousness grow at tomorrow's full use of these developments.

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Preservation of Russian Caviar by Canning[a]

A. S. LEVINE, C. R. FELLERS and R. R. BARTON

Department of Food Technology, University of Massachusetts, Amherst, Massachusetts[b]

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A detailed procedure is presented for preserving Russian caviar in hermetically sealed glass containers by a pasteurization process. The preserved product retains original quality as indicated by little or no change in color, texture, and flavor. The heat treatment is adequate to prevent spoilage through microbial action.

Introduction

Caviar, in the strictest sense of the word, is the roe of a species of sturgeon *(Acipenser sp.)* prepared in various grades as an article of food *(3)*. As little salt as possible is used in the manufacture of the higher grades. In this investigation two grades of caviar were used: Osetrina and Sevruga, the former being a slightly higher grade than the latter. There is very little published information on the packaging and processing of Russian caviar in this country. Jarvis *(2)* calls attention to the fact that some publications stating the process as 50 minutes at 250° F. (121° C.) are entirely in error. His experimental packs indicated that heating under pressure coagulated and hardened the sturgeon roe to such an extent as to make the eggs inedible. In the same bulletin published in 1943 *(2)* he also states that "accurate data on processing are not available at present. It is reported that caviar is pasteurized for about 60 minutes at 170° to 180° F. (77° to 82° C.) but this has not been verified." A paper dealing with the one-ounce size vacuum-sealed jar of a popular brand of Russian caviar made no mention of processing time or temperature *(1)*. Von Loesecke *(4)* mentions only that the caviar is packed in the final container (usually glass), sealed, and processed. Information gleaned from various sources in the industry was either qualified with statements that occasional spoilage troubles were encountered or that recommendations were not checked with experimental packs.

The results of this investigation indicated that it is possible to preserve Osetrina and Sevruga grades of Russian caviar in hermetically sealed glass containers by a pasteurization process that would result in a product showing little or no loss in quality as shown by color, texture, and flavor changes. Samples have been held at 80° F. (26.5° C.) for over two years without spoilage through microbial action.

A panel of competent judges, all acquainted with European caviars, have declared these experimental packs to be superior to three types of Russian caviar packed in one-ounce glass containers and purchased in the local markets.

Chemical Studies

The following is a chemical analysis of the two types of Russian caviar studied:

	Sevruga	Osetrina
Water	44.89%	47.06%
Protein	23.32%	28.29%
Fat	15.29%	16.04%
Ash	7.54%	5.78%
Salt (NaCl)	6.96%	5.26%
pH	5.3	5.1

Urotropin (hexamethylenetetramine), a preservative commonly used in Russian caviar for European consumption but not permitted in the United States, was not present in either sample. This preservative was also absent in three commercial samples of Russian caviar packed in one-ounce jars.

[a] Thanks are due to the Schenley Distillers Corp., N. Y. C., for liberal quantities of authentic Russian caviar.

[b] Contribution No. 719 Massachusetts Agricultural Experiment Station, Amherst.

Preliminary pasteurization studies indicated that caviar may be heated at temperatures lower than 160° F. (71° C.) with no marked change in appearance and flavor. Temperatures higher than 160° F. (71° C.) adversely affected the texture of the caviar as well as imparted a coagulated or "cooked" appearance to the product. To obviate or reduce heat treatment an attempt was made to lower the pH by the addition of acetic and citric acids. The natural buffering action of the caviar necessitated the addition of comparatively large amounts of each acid in order to lower the pH to 4.5 (from 5.1 and 5.3) and undesirable flavor changes occurred. The addition of acetic acid without heat also coagulated the caviar protein. Sodium benzoate and benzoic acid, two preservatives permitted in the U. S., also caused undesirable flavor changes when used in concentrations of 0.1%.

Microbiological Studies [c]

The susceptibility of caviar protein to coagulation by heat must always be considered, but essentially this is a bacteriological problem and should be investigated as such.

Fresh refrigerated samples of caviar were examined for total numbers of bacteria growing on Tryptone Glucose Extract Agar and incubated at 98.6° F. (37° C.) and 68° F. (20° C.) Both types of caviar showed higher counts when incubated at the lower temperature. In general, Osetrina had a count of over 17 million cells per gram and the Sevruga caviar had over nine million cells per gram. These counts are consistent with the salt contents of the two samples but are excessively high. It must be remembered that these samples had been held fresh under refrigerated conditions for an indeterminate length of time as they originally came from Russia.

Aerobic bacteria present were largely Gram-positive cocci and rods and the microorganisms cultured anerobically were found to be morphologically similar. Coliform organisms were found in one sample of fresh caviar but were totally lacking in the processed samples. Proteolytic types were completely eliminated by pasteurizing. No intestinal pathogens were found in either fresh or processed caviar. The selective medium used for the detection of intestinal pathogens was Bacto-SS Agar. A small number of yeasts was present in the fresh Sevruga samples.

Processing Studies

The microorganisms naturally present in caviar such as yeasts, molds, short rods, and cocci were destroyed at a temperature of 145° F. (63° C.). Preliminary tests indicated that 155° F. (68° C.) was the maximum processing temperature at which quality could be maintained.

Heat penetration studies were made on three sizes of glass jars commonly used for caviar: one-ounce squat jar, two-ounce tall jar (conical shape) and four-ounce tall jar (conical shape). For the "cold point" of jars packed with caviar to reach a temperature of 145° F. (63° C.) when immersed in a water bath of 155° F. (68° C.), 15 minutes were required for the one-ounce jars, 24 minutes for the two-ounce jars, and 33 minutes for the four-ounce jars. The initial temperature of the caviar was 70° F. (21° C.). The predominant microorganism isolated from the fresh caviar, a diplococcus with ill-defined characteristics, was killed in ten minutes at 145° F. (63° C.). Greater reliance is placed on the fact that no microorganisms developed in experimental packs after long incubation at room temperature. Samples have been held for as long as 30 months at 80° F. (27° C.) with no microbial spoilage.

Recommended Procedure

The following procedure is satisfactory for packing Sevruga and Osetrina Russian caviar similar in composition and microbial flora to the samples used in this investigation:

1. Clean, sterilize and drain the glass jars and caps to be used. Hot jars must not be used as they will coagulate the caviar protein.
2. Fill the cooled jars with caviar and cap immediately. Seal the containers under a mechanical vacuum (vacuum-sealer) of 20 inches or higher. The vapor-vacuum type sealer which employes a steam jet cannot be used as it coagulates the surface caviar.
3. Pasteurize in a water bath at 155° F. (68° C.) for the following times:

Size of Container	Time
1 oz.	30 min.
2 oz.	45 min.
4 oz.	60 min.

The water bath must not be below 155° F. (68° C.) nor above 160° F. (71° C.). The caviar, if filled from trays will have a temperature from 50° to 60° F. (10° to 16° C.) which is satisfactory.

4. Remove jars from water bath and cool immediately in cold water or a cold spray.

This procedure would lend itself well to the use of a continuous water bath processing unit and cooling line.

Storage

Cool storage of the packed product at 60° F. (15.5° C.) or lower is desirable and maintains for many months the freshness and quality of the canned caviar. The fresh salted caviar for packing should be held at a temperature as close to 32° F. (0° C.) as possible.

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[c] Credit is due to Margaret Dean, formerly of the U. S. Fish and Wildlife Service, Technological Laboratory, College Park, Md., for some of the bacteriological data.

Tyrosine Crystals in Glass-Packed Caviar[a]

A. PATRON, A. S. LEVINE, AND C. R. FELLERS
Department of Food Technology, University of Massachusetts, Amherst, Massachusetts

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Glass-packed Russian caviar held in storage for a long time develops whitish irregular spots which resemble mold growth and detract from the product's appearance.

This investigation shows that these spots consist of crystalline tyrosine. The probable causes of this type of chemical spoilage are discussed in this paper.

Introduction

Russian caviar packed in this laboratory *(6)* in one- and four-ounce glass jars, sealed with a vacuum type of closure, and pasteurized, was observed to develop whitish irregular spots on long storage at room temperature (see Figure 1). Their appearance seemed to indicate biological spoilage but later investigation showed that there was no active growth of any microorganisms including molds. Commercial jars of caviar were also observed to have developed these undesirable spots.

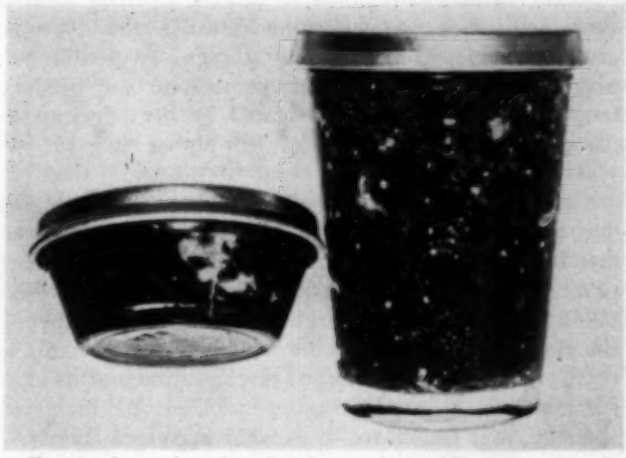

FIG. 1. Jars of caviar showing typical white spots that developed during storage.

As a general rule, the various types of caviar are characterized by a high protein content. Levine, Fellers, and Barton *(6)* showed that the caviar used in this investigation had a protein content of 23.32 percent for the Sevruga type and 28.29 percent for the Osetrina type.

Buttenberg *(2)* reported that the total acidity in commercial samples of caviar in Germany ranged from 4.5 to 8.4 (expressed as milliliters of normal alkali per 100 grams). These figures are fairly high, but Hinard *(3)* gave similar figures for commercial products from Russia, Rumania, and France.

Koenig and Grossfeld *(5)* investigated particularly the nature of the proteins in caviar and found that caviar is rich in ichthulin, a protein insoluble in water and in sodium chloride solutions. They also found that tyrosine is the prevalent amino acid in ichthulin.

Hinard *(3)* stated that perfectly fresh sturgeon roe is odorless and tasteless, and the flavor which develops later is the result of a slight "fermentation" or "ripening" action.

However, if chemical changes progress too far, the product is likely to develop an unpleasant fishy flavor, and become tart and rancid. Although very little is known about the chemical changes that take place during the ripening period, Hinard *(3)* observed a progressive increase in total acidity and free amino acid content. He concluded that these changes seemed to represent more of an enzymatic fermentation than a microbiological fermentation.

Experimental

Physical and Chemical Examination of Crystals. The samples analyzed in this investigation were bulk Osetrina and Sevruga types of Russian caviar, packed by the authors in one- and four-ounce glass jars, sealed hermetically, and pasteurized. All samples were of the grain type and included a commercial sample which also showed this marked discoloration. The jars packed in this laboratory were held over two years at a temperature of 80° F. (26.5° C.).

Numerous cream-colored spots of one to four millimeters in diameter had developed on storage and could be seen on the inside wall of the glass. These discolorations were also observed throughout the product after the jars were opened. They were sufficiently numerous and noticeable to give the product an objectionable appearance. Taste tests indicated that the discolored product was fairly well accepted in spite of a slight fishy flavor which was probably caused by long storage at a fairly high temperature.

The first impression was that these spots were the result of a biological development, possibly a mold growth. When observed under low magnification, the spots displayed a crystal-like structure; more specifically, long thin needles arranged radiantly. The shape and arrangement of these crystals resembled the structure of tyrosine crystals as reported and pictured by Koenig and Grossfeld *(5)*, and obtained by chemical hydrolysis during the analysis of carp roe.

Examination of some of the cream-colored material under polarized light confirmed the crystalline structure and it was found to be similar to pure tyrosine crystals in appearance and optical properties when viewed between crossed Nicol prisms (see Figure 2).

Some crystals fairly free of adhering caviar material could be obtained from the samples by scraping carefully. These crystals were washed with benzene and alcohol to remove traces of adhering material including fat. Tests showed that these crystals were very slightly

[a] Contribution No. 270 of the Massachusetts Agricultural Experiment Station.

FIG. 2. Tyrosine crystals isolated from glass-packed caviar. x100. In polarized light.

soluble in water, and insoluble in ether, ethyl alcohol, and benzene, but quite soluble in a dilute solution of sodium hydroxide. Pure l-tyrosine is soluble in alkali, its solubility in water is 0.048 per cent at 77° F. (25° C.), and is practically insoluble in the other solvents used above.

These preliminary observations indicated that these crystals might be tyrosine that had crystallized out of the stored caviar. Some additional tests were conducted on the washed crystals to confirm identification. The needles on ignition gave off a characteristic odor of burning hair which indicates the presence of proteic material. No ash residue remained, showing that it was purely organic matter. The presence of nitrogen was also indicated by a strongly positive Lassaigne test, and the presence of a phenolic derivative was shown by a positive Millon test. Tyrosine is the only common amino acid containing a phenol group. However, the biuret reaction was repeatedly negative indicating the absence of peptide linkage which showed that the material consisted of a single amino acid. The alpha-nitroso-beta-naphthol reaction which is specific for tyrosine was positive.

The presence of tyrosine crystals in fish pastes has been reported briefly by Burrell *(1)* although no experimental evidence was furnished.

Chemical Examination of Caviar. At this point in the investigation, it was considered of interest to know how the product happened to be so rich in tyrosine that it had crystallized out.

Hinard *(3)* observed an hydrolysis of both the protein and the fat of caviar during ripening. He devised a technique, derived from the formol titration method, for determining the acidity and the amino acid content of caviar. From a number of analyses of good caviar of known freshness, Hinard tried to set some standards; he felt that the acidity should not be over 2 ml. of normal alkali per 100 grams and formol titratable nitrogen not over 0.05 percent.

Therefore, the acidity and the free amino groups were determined according to his technique as follows:

A sample consisting of 20 grams of caviar was thoroughly ground in a mortar, completely crushing the eggs and disintegrating the membranes, 50 ml. of water were slowly added, and then 50 ml. of alcohol under continuous stirring. The mixture was introduced into a flask and vigorously shaken. After a few minutes' standing, the cloudy mixture was filtered through paper, yielding a brilliantly clear liquid with slight yellowish coloration. A mixture of 25 ml. of this liquid and 75 ml. of water, was titrated with standardized sodium hydroxide solution, using phenolphthalein as an indicator, until a definite pink coloration appeared. This first result represented the total acidity of the product.

To this neutralized solution 10 ml. of a neutral 40 percent formol solution were added and the formol titration was completed as usual. In the above procedure the addition of 75 ml. of water is necessary to minimize the error caused by the presence of alcohol.

Results obtained from two different samples of caviar were as follows: 10.8 and 16.3 ml. N-alkali per 100 grams for total acidity and 0.227 per cent and 0.283 per cent amino nitrogen respectively (formal titration), These results are higher than the upper limits recommended by Hinard *(3)*, and even higher than the results obtained by Buttenberg *(2)* on commercial samples.

Discussion

Whether or not these jars had been packed with overripened "caviar," it is probable that the ripening process continued after pasteurization because the tyrosine crystals developed during long storage. A possible explanation for this is that the proteolytic and lipolytic enzymes had not been inactivated by heat during the pasteurization process, which is not higher than 155° F. (68.5° C.).

Cold storage of processed caviar seems to be highly desirable to retard ripening and, in fact, was recommended by Jacobsen *(4)*. It is possible that such a practice would reduce considerably the autolysis which takes place at room temperature, and, as a consequence, the crystallization of tyrosine would not be so likely to occur. Because of the marked discoloration and its close resemblance in the consumer package to biological spoilage, it is important to prevent or retard this tyrosine crystallization. This objectionable development has been observed several times in present-day commercial samples of caviar.

The presence of these spots also indicates that the product is old and has been improperly stored for many months.

Summary

Some samples of glass-packed caviar stored for about two years at room temperature underwent a crystallization of tyrosine throughout the product. The examination of two samples showed an abnormally high total acidity and free amino nitrogen content. It is suggested that pasteurized samples of caviar be stored at cool temperatures to prevent this unsightly condition.

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Sugars of Citrus Juices[a]

R. M. McCREADY, E. D. WALTER, AND W. D. MACLAY
Western Regional Research Laboratory,[b] Albany, California

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Analyses for sugars in lemon, orange, and grapefruit juices by paper chromatography show the presence of only sucrose, glucose, and fructose. Their identities were confirmed by independent means. Fermentation of the citrus juices with Torula monosa after inversion of sucrose removes all traces of sugars. Nearly 100% of the solids from deionized lyophilized Valencia orange juice is accounted for by analysis.

Because of the lack of qualitative and quantitative knowledge of the sugars in citrus fruits, it was considered worth while to use the method of paper chromatography *(6, 7)* in the investigation of this problem. In this work, glucose, fructose, and sucrose were identified by their movements and color reactions on paper. Sucrose was crystallized from orange juice. Glucose was oxidized to gluconic acid and identified as its potassium salt.

Quantitative and qualitative analyses for sugars in fresh juices before and after fermentation with *Torula monosa* and after acid hydrolysis are presented. The analysis of deionized and lyophilized orange juice solids after removal of alcohol-insoluble barium salts and treatment with ion exchangers is given.

Experimental

Methods. Lemons (Eureka, California grown), grapefruit (Marsh, Arizona grown), and oranges (Valencia, California grown) were burred to remove the juice from the fruits. The juice was strained through cheesecloth, heated rapidly to 85° C. for one minute, and cooled quickly to room temperature. The insoluble solids were removed by filtration through paper coated with a fine grade of processed diatomaceous silica filter aid. This juice was used for the qualitative analysis for sugars by paper chromatography.

For the preparation of lyophilized orange solids, juice from the burring machine was mixed with barium carbonate, strained through cheesecloth, and then filtered through coarse filter paper. Three volumes of 95% ethanol were then added and the barium salts removed by filtration. The filtrate was concentrated to the original volume *in vacuo*, decolorized with about 0.2% charcoal, and filtered using processed diatomaceous silica filter aid. The filtrate was treated with ion exchange materials to remove inorganic constituents and this salt-free solution was frozen in solid carbon dioxide isopropyl alcohol mixture, and evaporated to dryness *in vacuo*. Lyophilization produced a light fluffy and very hygroscopic powder suitable for chemical analysis.

The soluble solids content of the juice was determined by the refractometer. Reducing sugars and total sugars after invertase hydrolysis were determined, without charcoal treatment, by the method of Hassid *(4)*. Glucose was determined on the dried orange solids by a hypoiodite method *(11)*, and fructose was obtained as the difference between total reducing sugars and glucose. Hesperidin was determined by the method of Davis *(3)*.

Qualitative identification of sugars was done by the method of paper chromatography *(6, 7)*. Aqueous *n*-butanol was used as the moving solvent. The temperature was 25° C. and the time for development of the chromatogram was about 20 hours. After the paper sheets had been dried at 105° C. for 10 minutes, they were sprayed with Sumner and Sislers' dinitrosalicylate sugar reagent *(9)* and heated again for 10 minutes at 105° C. The reducing sugars appeared as brown spots on a yellow background. The positions of sucrose and fructose were revealed by pink spots on a white background after the dried sheets were sprayed with 0.1% resorcinol in 1*N* hydrochloric acid solution and heated at 80° C. for 10 minutes. The sugars on the paper sheets were identified by authentic samples of known sugars as controls. This method is more dependable than relying on "R_f" values alone.

Sugars were fermented from the juices after invertase hydrolysis at pH of 5.0. Packed washed cell suspensions of *Torula monosa* in phosphate buffer were added to 100-ml. portions of citrus juices at pH 5.0. An amount equivalent to 1.0 ml. of cells was added to lemon, 2.5 ml. to grapefruit, and 5.0 ml. to the orange juice. Different quantities of cell suspension were required to ferment the sugars from these juices in less than 40 hours.

One hundred milliliters of the sugar-free juices were concentrated *in vacuo* to 25 ml. each and tested qualitatively for sugars before and after hydrolysis in boil-1*N* hydrochloric acid for 1 hour.

Results

The results of sugar analyses on single strength juices in Table 1 are in agreement with those of Chatfield and McLaughlin *(1)*, Roberts and Gaddum *(8)*, Widdowson and McCance *(10)*, and Curl and Veldhuis *(2)*. The values for reducing sugars and total sugars have been corrected for non-sugar reducing substances that react with ferricyanide under the conditions of sugar analysis.

TABLE 1
Sugars in Filtered Single-Strength Citrus Juices

Juice	Solids, %	Sugars		Flavanone *(3)*, %
		Reducing, as glucose, %	Total, %	
Lemon	7.5	0.94	0.94	0.046
Grapefruit	8.0	4.36	5.00	0.038
Orange	12.5	5.12	9.82	0.08

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 12, 1949.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

The qualitative tests for sugars are presented in Table 2. Paper chromatograms under the conditions used are capable of detecting concentrations of sugars down to 0.05%; therefore glucose, fructose, and sucrose are present in concentrations above this level. It is of interest to note that lemon juice contains a trace of sucrose which is not detected by chemical analysis.

TABLE 2

Qualitative Tests for Sugars in Citrus Juices by Paper Chromatography

Juice	Sugars present			
	Single strength	Single strength, after fermentation	Four-fold concentrate after fermentation	
			Before acid hydrolysis	After acid hydrolysis
Lemon (Eureka, Calif. grown)	Glucose, fructose, and a trace of sucrose	None	None	None
Grapefruit (Marsh Arizona grown)	Glucose, fructose, and sucrose	None	None	Trace of galactose and rhamnose [a]
Orange (Valencia, Calif. grown)	Glucose, fructose, and sucrose	None	None	Trace of galactose, glucose, and rhamnose

[a] Very faint traces of sugars represented by concentrations of 0.05% or less, the lower limit for qualitative tests for sugars under these conditions.

After the fermented sugar-free juices were concentrated and hydrolyzed by boiling dilute hydrochloric acid, traces of galactose and rhamnose in grapefruit, and galactose, glucose, and rhamnose in orange were detected, thus demonstrating the presence of small amounts of acid-hydrolyzable glycosides. The detection of trace quantities of mannose in the presence of large amounts of fructose is difficult, since the movements of these sugars are closely similar on the chromatograms, and both are fermented by *Torula monosa*. No mannose phenylhydrazone was formed from the juices under conditions used for the identification of mannose. Table 3 contains results of the analysis of deionized Valencia orange solids, where nearly 100% of the material is accounted for by these analyses.

TABLE 3

Analyses of Deionized and Lyophilized Valencia Orange Juice Solids

	1947 fruit %	1946 fruit %
Sucrose	52.8	41.0
Glucose	20.0	21.0
Fructose	16.0	21.0
Hesperidin	0.60	0.60
Protein	2.3	1.9
Ash	0.10	0.10
Moisture	9.2	12.6
Total accounted for	101.0	98.2

The presence of sucrose is confirmed by the refractive indices of its crystals obtained from orange juice ($N_\alpha = 1.540$, $N_\beta = 1.567$, $N_\gamma = 1.572$). Glucose was confimed by oxidizing the liquor from the sucrose crystallization using the Moore and Link *(5)* method. The resulting gluconic acid was converted to potassium gluconate, melting at 180° C., identical with that of an authentic specimen.

Summary

Paper chromatography was used to demonstrate the presence of glucose, fructose, and sucrose in citrus juices. The presence of sucrose was confirmed by its crystallization and optical properties. Glucose was confirmed by its conversion to potassium gluconate. Fructose was identified by its color reactions with resorcinol, and its stability to hypoiodite.

Fermentation with *Torula monosa* completely removes sugars from citrus juices. Trace quantities of acid-hydrolyzable glycosides, containing galactose and rhamnose in grapefruit and these plus glucose in orange juice were detected by paper chromatography. Nearly 100% of dry deionized Valencia orange solids was accounted for by chemical analysis.

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Methods for Determining the Incidence of Putrefactive Anaerobic Spores in Meat Products[a, b, c]

MARTIN V. BURKE, KEITH H. STEINKRAUS AND JOHN C. AYRES
Bacteriology Section, Iowa Agricultural Experiment Station, Ames, Iowa

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Introduction

In view of the low numbers of putrefactive anaerobic spores in commercial meat products, a most probable numbers technique was found to be the most satisfactory method for determining spore incidence. This method was used to make counts of samplings from a large number of fresh, cured and processed samplings of pork luncheon meat. At the same time comparison studies were made of three liquid anaerobic media selected for subculture.

Sterilization periods advocated by laboratories connected with the canning industry assume the presence of heavy loads of putrefactive spores and spoilage organisms in meat products prior to heat processing. On the other hand, it is known that lower sterilizing values result in canned meats having more acceptable flavor and possibly better nutritive values as well. Since most non-acid canned foods are processed to sterilizing values in excess of $F_0 = 2.78$, Ecklund, et al. *(2)* state that packers of luncheon meat using sterilizing values lower than this figure rely to some extent on the bacteriostatic effect of curing agents and a rigid program of plant sanitation to prevent losses through spoilage. The possibility that the spores associated with the product can germinate must be fully considered before any lower sterilizing value can be adopted.

It would be desirable to have quantitative data on the incidence of putrefactive anaerobic spores in meat because of their role not only in spoilage but in food poisoning as well. Adajian and Gardner *(1)* noted that the packer is limited to those cooking processes which sterilize the product but do not injure its appetizing quality. They emphasized that packers must compute the thermal process to destroy the normal amount of contamination and then add a minimum safety factor. Compilation of data pertaining to the incidence of putrefactive anaerobic spores in meat might be taken as a base line from which adequate processes could be derived. Only then would a logical investigation be possible which could lower thermal processes to a level that would: (1) yield a meat with maximum nutritional and organoleptic qualities and at the same time (2) destroy *Clostridium botulinum* spores, and (3) give a minimum spoilage of the finished product.

Harriman, et al. *(5)*, using liver infusion broth as a substrate, indicated spore loads to be very light in the plant in which their study was conducted. Whether this condition prevails throughout the industry is not known. The question regarding the incidence of viable anaerobes in fresh and in cured meat therefore is of great concern. Methods of sampling and media for subculture are of prime importance in obtaining satisfactory data regarding surviving organisms.

The primary objective of this investigation is to obtain an estimate of the normal load of putrefactive anaerobic spores present in a commercially-prepared comminuted meat. Secondarily, it is hoped that any data acquired here might be utilized as a partial basis for computation of more proper heat proceses. Stumbo, Gross and Vinton *(9)* stated the point quite well when they maintained that a convenient, inexpensive method was urgently needed for determining, by routine examination of the meat to be canned, the heat processes necessary to prevent spoilage of the canned product. Further, studies concerning the influence of various factors upon the thermal resistance and growth of bacteria in meat should aid materially in establishing heat processes that are sufficient to prevent spoilage of the canned product and that allow meats to be processed with a minimal loss of organoleptic and nutritional values.

Experimental

Meat samples were collected from 750 lb. lots of pork trimmings that had been mixed as thoroughly as commercially feasible. Plans called for sampling: (1) freshly ground pork trimmings, (2) cured but not heat processed product and (3) finished product. This sequence could be modified to include other desired points on the finishing line. One to two pound representative samples were obtained at each stage, reground through a KitchenAid K-4B meat grinder and mixed thoroughly for ten minutes with the KitchenAid mixer.

A sterile chamber (4′ x 4′ x 2′) similar in some details to that devised by Wise *(10)* was used during inoculation in order to reduce air-borne contamination (See Fig. 1). As this sterile case was part of a general hood system it contained ventilation outlets and a sliding glass front. The ventilation outlets were plugged with cotton and rubber gasket-material was fitted across the front between the frame and the movable window. One 15 watt germicidal ultra-violet lamp was installed on either side of the chamber and during a "run" remained lit except when meat or organisms were exposed. Entrance to the sterile-case was gained by inserting the arms through the sleeves of a sterile flannel apron fitted to the lower part of the window. Each day, before the start of an experiment, the chamber was swabbed with mercuric chloride solution.

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FIG. 1. Inoculating chamber used for stuffing meat into tubes.

Yesair's liquid pork extraction, beef liver infusion and Difco thioglycollate media were compared in an attempt to determine if one of the substrates consistently would give the highest recoveries of putrefactive anaerobic spores from meat products. To Yesair's pork extraction, 0.1% soluble starch and 0.5% sodium thioglycollate were added. This modified Yesair's medium duplicated the substrate used by Wynne and Foster *(11)*. Beef liver infusion medium was prepared in accordance with directions given in the Manual of Methods for Pure Culture Study of Bacteria (1946). Difco thioglycollate medium (Linden's modification) was used in the commercially prepared form except that 0.1% soluble starch was added.

Two methods, one gravimetric and the other volumetric, were used with the Yesair's medium. Before making gravimetric determinations, sterilized test tubes were weighed on a torsion balance. Then twenty to sixty replicate amounts of ten, one and one-tenth gram of meat were transferred to the tared tubes. A larger number of replicate tubes were employed when greater precision was desired. A calking gun was used to transfer the proper amounts of meat. After the tubed meat had been weighed to an accuracy of $\pm$ 5%, sterile medium was added at a ratio of ten parts of medium to one part meat.

Care was taken to avoid entrapping air in the tubed meat. Attachments of the calking gun were designed to reach to the bottom of the different sized tubes. This gave a more solid pack and tended to prevent meat from remaining on the sides of the container. Prior to addition of the medium, each tube was sharply shaken to force air bubbles from the meat.

Later, a volumetric method, wherein 70 g. of meat were mixed with 280 ml. of physiological saline (0.85% NaCl) for ten minutes in a Waring Blendor, was employed rather than the tedious gravimetric procedure. Quantities of meat equivalent to those weighed were prepared by addition of appropriate amounts of the mixture to the Yesair's medium so as to give a final ratio of one part meat to ten parts liquid.

For tests with Difco thioglycollate or beef liver infusion medium, mixing was performed in the same manner as for the volumetric method given above. In both cases five replicate 5 ml., 0.5 ml., or 0.05 ml. quantities of the mixture were added to tubes containing ten ml. of the sterile medium.

All tubes were heated to 80° C. for twenty minutes to destroy vegetative cells. After heating, the Yesair's pork extraction tubes were placed under anaerobic conditions using the technique described by Ogilvy, Burke and Ayres *(7)*, (Figure 2). The tubes containing meat in Difco thioglycollate medium or beef liver infusion were sealed with a layer of paraffin pipetted directly over the surface of the substrate.

A number of experimental batches of fresh, cured unprocessed, and heat processed meat were inoculated with a standard spore suspension of the test organism, Putrefactive Anaerobe No. 3679. A load of one spore per gram generally was inoculated as this was considered to approximate conditions normally existing in meat. Similar but uninoculated meat samples served as controls. After thorough mixing twenty samples each of two, one and one-tenth gram were transferred to tubes to give a total of sixty tubes in the control and sixty tubes in the inoculated run.

Tubes were incubated for a period of ten days at 37° C. At the end of that time they were observed for meat digestion, color change, putrefactive odor, gas production and cloudiness. Following organoleptic evaluation, 0.1 ml. of the tubed meat-medium mixture was subcultured to fresh medium and the subculture tubes held at 80° C. for twenty minutes to eliminate possible vegetative cells that might have germinated from aerobic spores after the first heat treatment. Subculture tubes were incubated at 37° C. for an additional four days. At the end of this period the tubes were observed for growth and counts obtained by (1) calculation using Halvorson and Ziegler's *(3)* formulae or by (2) comparison with Hoskin's *(6)* "most proba-

FIG. 2. Anaerobic jar containing tubes of meat and medium.

ble numbers" tables. Microscopic examination was made of positive and doubtful tubes.

Results and Discussion

Methods of sampling and media used for subculture are of considerable importance in determining the incidence of putrefactive anaerobic spores in meat products. Preliminary trials with various methods indicated that only the dilution methods provided a convenient means for counting anaerobic bacterial spores in comminuted meat. The necessity for determining viable spores, the low spore populations and the character of the meat excluded the possibility of using direct or proportional microscopic counts, turbidimetric readings or other methods proposed for enumeration of microorganisms by indirect means.

Representative methods employing liquid or solid substrates were compared. Several anaerobic plating methods, including a modified Krumwiede-Pratt plate and a slide-coverglass technic developed by Steinkraus and Ayres *(8)* did provide methods for enumerating viable anaerobic spores on the basis of colony formation. However, the spore loads of meat products examined in this study were so low that anaerobic plate counts were not thought to be reliable.

From a number of liquid media, three were selected for comparison studies to determine whether one of the substrates yielded consistently higher recoveries than were obtained by either of the other two. One of the three media chosen, a modified Yesair's liquid pork extraction, was found by Wynne and Foster *(11)* to give better germination of anaerobic spores than did any of the other media that they tested. The second medium, beef liver infusion, has been employed by workers *(5)* in determining base line data on the presence of heat resistant putrefactive spores in meat products. The modified Difco thioglycollate medium was selected as the third substrate in view of the excellent results obtained with it in this laboratory.

Table 1 shows a larger number of positive tubes in 0.1 g. samples than in 1 g. or 10 g. samples of the same meat. Ordinarily a hundred-fold decrease would be expected from 10 g. to 0.1 g. Errors in sampling might account for some variation, but with the large number of subcultures, such deviation would be lessened. Also, since a standardized procedure was adopted, errors in sampling were minimized.

Another possibility for such discrepancy was dilution of the inhibitive factor or factors upon addition of larger amounts of medium. The curing agents involved previously have been shown to exert some bacteriostatic effect in prepared media. In Table 2, it may be seen that the number of positive samples increased with greater dilution of the meat. No significant change was noted beyond a meat-medium ratio of 1:10, which was in good agreement with Yesair and Cameron *(12, 13)* who found that diluting the cured meat with eight volumes of liquid (medium) apparently reduced the effective salt concentration below the stage of inhibition. Less inhibition was apparent in the fresh uncooked trimmings than in the cured product as indicated by the data shown in Table 3.

TABLE 1

Inhibitory Effect Shown in MPN Determination of Anaerobic Spores in Cured, Unprocessed Meat

	10 gm. sample 1:1 meat-medium ratio	1 gm. sample 1:1 meat-medium ratio	0.1 gm. sample 1:10 meat-medium ratio
Number of tubes initially incubated	60	60	60
Number of tubes showing putrefactive anaerobic spores	12	3	13
Probable number of positive tubes expected with no inhibition in 0.1 gm. sample	60	60	13

TABLE 2

Effect of Cured Meat-Medium Ratio on Germination of Putrefactive Anaerobic Spores

	Trial I				Trial II			
Dilution ratio, meat to medium	1:10	1:5	1:3	1:1	1:20	1:15	1:10	1:1
Number of 1 gm. samples tubed	40	40	40	40	20	20	20	20
Number of tubes showing putrefactive anaerobic spores	24	17	6	3	9	10	12	0
% positive tubes	60	42.5	15	7.5	45	50	60	0

TABLE 3

Effect of Fresh Pork to Medium Ratio on Germination of Anaerobic Spores

Dilution ratio, meat to medium	1:20	1:15	1:10	1:5	1:3	1:1
Total number of one-gram samples of fresh pork	40	40	40	40	40	40
Number of samples showing putrefactive anaerobic spores	14	24	16	18	14	8
% positive samples	35	60	40	45	35	20

Results in Table 4 indicate a fairly wide variation in spore loads as determined by the individual medium used to test that product. There are a number of possible explanations for the differences in count. Yesair's medium was used by one worker while the liver infusion and Difco thioglycollate substrates were employed by another. Twenty replicate tubes were utilized at each of the three dilutions when determining probable numbers of spores in meat cultured in Yesair's pork extraction while only five replicates were used at each dilution when testing with either of the other two media.

Two factors govern the choice of the number of tubes of each dilution to be employed in each experiment.

TABLE 4

Comparative Spore Counts per 100 g. Luncheon Meat Inoculated into Yesair's Pork Extraction (Y), Beef Liver Infusion (L), or Difco Thioglycollate (D) Medium

Sample No.	Fresh Trimmings			Cured Trimmings			Heat Processed Product (165° F. for 3-4 hrs.)		
	Y	L	D	Y	L	D	Y	L	D
1	68	45	20	39	130	78	53	0	0
2	21	78	110	48	230	210	279	45	37
3	19	220	170	7	140	61	9	0	20
4	50	93	110	<1	0	20	325	45	20
5		230	251	5	40	0	3000	120	330
6		10	167		2400	170	1600	460	170
7		78	0	47	110	40			
8	*				0	20			

*Eight additional individual runs were made with Yesair's medium using fresh trimmings. Counts were: 21, 12, 44, 65, 14, 8, 66, 16.

First, greater precision is obtained with increased numbers of tubes. Second, a sufficient number of negative tubes must be present to indicate any difference in count between the control and the inoculated meat. Halvorson and Ziegler *(4)* have shown that with twenty tubes in each of three dilutions, deviations from the mean which will include 97% of the data are respectively 48% below and 75% above, while, with five tubes, deviations of as much as 70% below and 260% above the mean may be expected.

The mixing operation was considered sufficient to give a homogenous sample by macroscopic standards. If spores localize in definite components of the meat (e.g., in the fat) or in certain areas, adequate mixing would require more serious study and special consideration.

The percentage distribution frequency of spore counts obtained with the three media (See Table 5)

TABLE 5

Percentage Distribution Frequency of Spore Counts per 100 g. Luncheon Meat Inoculated into Yesair's Pork Extraction (Y), Beef Liver Infusion (L), or Difco Thioglycollate (D) Mediums

No. of spores per 100 g. meat	Fresh Trimmings			Cured Trimmings			Heat Processed Product		
	Y	L	D	Y	L	D	Y	L	D
0-10	8	0	22	50	38	11	17	45	22
10-100	92	67	22	50	12	56	58	33	45
>100	0	33	56	0	50	33	25	22	33

failed to demonstrate better germination of spores with any of the media employed. It should be mentioned that, while the media were equally satisfactory, Yesair's pork extraction and beef liver infusion were subject to the following drawbacks: (1) preparation tedious and time consuming, (2) difficulty of removing all or equivalent amounts of fat, (3) variations in meat available and (4) presence of a precipitate after autoclaving. On the other hand, Foster and Wynne *(11)* have indicated that different batch lots of commercially prepared media may also vary in composition. Insofar as comparative levels of fat or other components are concerned from animal to animal, less variation would be expected if large amounts were prepared.

It was not practical to collect samples from the same trimmings in the fresh, the cured and the processed state. Results shown in Table 5, therefore, do not indicate reduction or growth of bacteria during the various stages of processing.

Since these results represent inoculation and study of over 5600 tubes for a period of six months, they give a general indication of the number of putrefactive anaerobic spores that were recovered from fresh, cured and processed trimmings from one commercial meat-packing plant. It is interesting to note that with two exceptions the largest percentage of probable spore counts was less than one per gram. Fresh trimmings cultured in Difco thioglycollate and cured trimmings inoculated in beef liver infusion gave 56% and 50%, respectively, of their spore loads in the range 110-2400 per one hundred grams or 1.1-24 per gram. The largest number of organisms recovered in this study, as determined from positive tubes of Yesair's pork extraction medium, represented 30 spores per gram.

In a further attempt to determine the suitability of the volumetric method for spore counting and recovery, a number of determinations were made on fresh, cured and processed luncheon meat to which a definite load of P. A. 3679 spores were added. In these studies, only slightly better recoveries were reported for the gravimetric procedures than for the volumetric method. The average percentage recovery of artificially inoculated spores of P. A. 3679 from fresh trimmings was 73.5% while from cured trimmings it was 40.2% and from the commercially sterilized 12 oz. pork luncheon tins it was 96%. As the load of spores introduced was rather small, there was rather wide variation in percentage recoveries obtained among individual samplings.

Summary and Conclusions

An investigation was made to develop a convenient method for determining the incidence of putrefactive anaerobic spores in a comminuted meat product.

Dilution of the cured product with ten volumes of medium lowered the effective concentration of the curing salts to a point below which spore germination was not inhibited by these agents. With this as a basis, two methods were developed for determining the incidence of these spores in meat. One method used was gravimetric while the other was volumetric. In the ranges investigated results by the two methods were comparable.

Counts were determined on the fresh, cured and processed pork luncheon meat produced by commercial meat-packing plants. Spore counts in the meat sampled during this study were so low that only a most probable numbers counting technique and liquid media gave reliable results. These counts were determined by comparison of positive tube combinations with formulae or charts provided for that purpose. Of the various media examined, three were selected for comparison studies to determine if one would consistently give highest spore recoveries from meat. No appreciable differences were shown.

To test the effectiveness of the counting methods, a standard spore suspension of P. A. 3679 was inoculated and mixed with pork luncheon meat. The level of inoculation was one spore per gram of meat, a load considered to represent conditions in this type product. Good recoveries were obtained.

A large number of determinations were made on samples of the fresh, cured and processed product. The average load of putrefactive anaerobic spores found in the product was less than one spore per gram. No appreciable difference was observed in the counts obtained from the three sampling points. The thermal process used in this study for six-pound cans of pork luncheon meat did not produce a noticeable decrease in the putrefactive anaerobic spore population.

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Industrial and Legal Viewpoints

Application of the Federal Food, Drug, and Cosmetic Act to Food Raw Materials Bearing Chemical Residues[a]

McKAY McKINNON, JR.
Food and Drug Administration, San Francisco, Calif.

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The history of chemical residue tolerances is recorded. Applicability of the Federal Food, Drug and Cosmetic Act to foods bearing toxic insecticidal residues is explained. Current data on chronic toxicities of new insecticides are included. The philosophy of consumer protection is discussed.

It is my purpose today to deal with the history of tolerances for chemical residues on foods as developed in enforcing the Food and Drugs Act of 1906 only to the extent that such data will provide illuminating background to those sections of the Food, Drug, and Cosmetic Act of 1938 which deal directly with the problem of added poisons in or on foods.

When the Food and Drugs Act of 1906 became law, there had not developed in the United States areas of concentrated production and intensive cultivation which subsequently enabled certain insect pests to outstrip their natural enemies and to wrest control of growing crops from the farmer and orchardist. By 1915, however, several bureaus of the United States Department of Agriculture recognized that remedial measures of extermination and prevention, rapidly becoming necessary on an organized basis to bring pomaceous fruits to successful harvest, might create a serious consumer hazard.

The Bureaus of Chemistry, Entomology and Plant Industry of the United States Department of Agriculture therefore embarked in 1915 on a long-range, joint study of spraying procedures to determine if spray schedules for various fruits and vegetables as recommended by the United States Department of Agriculture and its state counterparts could, if carefully followed, result in products which at market time, would bear poisonous chemical residues in quantities sufficient to endanger the health of consumers. These studies were published under the title "Poisonous Metals on Sprayed Fruits and Vegetables," Bulletin No. 1027, Bureau of Chemistry, U. S. Department of Agriculture, 1922. The report carried the conclusion that, provided proper schedules were followed, harvested crops should bear residues which would be insignificant from the standpoint of danger to health. Meantime, however, there were appearing on the market in ever increasing quantities foods which carried arsenical residues in hazardous amount. Since no comparable large scale studies had been made in the United States, the investigations, reports, and recommendations of the Royal Commission set up in England after the famous poison beer outbreak at the turn of the century, were studied and the Commission's tolerances were tentatively utilized as administrative guides in determining whether or not a product bearing residues would be deemed potentially dangerous to health.

Thus the tolerance for arsenic used by the U. S. Department of Agriculture at the outset was 0.01 gr./lb. as As_2O_3. This advisory figure was supplanted by a temporary tolerance announced by the Secretary of Agriculture in February 1927, of 0.025 gr./lb. as As_2O_3. The Secretary arrived at this figure in weighing the recommendations of a conference of distinguished technologists and physiological chemists, including such notables as Dr. Reid Hunt, Dr. Haven Emerson, and Dr. Carl Voegtlin, against what industry could accomplish at that time. The figure was set with the idea of reducing the tolerance as promptly as possible to the British figure or so-called World Tolerance of 0.01 gr./lb. Early in 1928 it became obvious that the World Tolerance could not be met with cleaning equipment then available, and the U. S. Department of Agriculture issued a notice to growers of apples and pears setting the tolerance for that year at 0.02 gr./lb. as As_2O_3, but indicating that the figure would be reduced to the so-called World Tolerance in 1929. In 1929 there was a considerable agitation for continuation of the 0.02

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figure, but in April the Department of Agriculture issued a notice setting the figure for 1929 at 0.017 gr./lb. as As_2O_3. In 1930 the figure was set at 0.015 gr./lb. In 1931 the figure was set at 0.012 gr./lb. with a statement that the World Tolerance for arsenic would be adopted in 1932, and in 1932, as forecast, the tolerance was set at 0.01 gr./lb. as As_2O_3 for that year.

Up to this time there had been no announcement on lead, although compounds of this metal were recognized as poisons and the arsenate had been widely used for spraying purposes. Further, as methods of cleaning fruit improved, particularly with the development of the Henry Washer, it was considered that an acid wash sufficient to strip arsenic from fruit would also strip lead. However, as alkali washes began to replace acid washes, it became necessary to consider the lead problem separately. In April 1933, the U. S. Department of Agriculture announced a tolerance for lead at 0.012 gr./lb. as Pb. and repeated the announcement of the arsenic tolerance of 0.01 gr./lb. By this time another weapon had been added to the orchardist's armamentarium and the Department also issued its first warning on the use of fluorine sprays. In June of 1933 the lead tolerance was relaxed to 0.02 gr./lb. as Pb. and a tolerance for fluorine was set at 0.01 gr./lb., with the warning that the lead tolerance would be reduced in 1934. In December of 1933 tolerances for the year 1934 were set as follows: Arsenic—0.01 gr./lb. as As_2O_3; Lead—0.019 gr./lb. as Pb.; Fluorine—0.01 gr./lb. as F. In January 1935 these tolerances were repeated with the exception that the tolerance for lead was reduced to 0.018 gr./lb. as Pb. These figures, 0.018 gr./lb. for lead, 0.01 gr./lb. for arsenic, and 0.01 gr./lb. for fluorine, remained in effect throughout the remaining life span of the Food and Drugs Act of 1906. All announcements were directed to growers of apples and pears and those fruits only. It should be kept in mind that these tolerances as announced by the Secretary of Agriculture from time to time, and as utilized in the enforcement of the Food and Drugs Act of 1906, were advisory only. In every contested action involving added poisons, such as poisonous residues on apples and pears, it was necessary to demonstrate in civil cases by a preponderance of evidence, and in criminal cases beyond a reasonable doubt, that quantities of poisonous residues in excess of these figures were in fact potentially dangerous to health. In assuming the burden of proof in this field, the agency enforcing the Food and Drugs Act of 1906 took as its authority for extending the limited protection afforded by that law, the opinion of the Supreme Court of the United States in the Lexington Mill and Elevator case (232 U.S. 399.) In the first seizure to be contested, apples and pears were involved. The Government in 1926 seized several thousand boxes of these fruits, shipped from Oregon to Illinois, alleging that they bore excessive quantities of arsenic compounds and lead compounds which rendered the articles potentially injurious to health. In turning this case over to the jury the Court, in harmony with the Lexington Mill and Elevator case, instructed the jury as follows:

"It is not required that the article of food containing added poisonous or added deleterious ingredients must affect the public health and it is not incumbent upon the Government, in order to make out a case, to establish that fact. The Act has placed upon the Government the burden of establishing, in order to secure a verdict of condemnation under this statute, that the added poisonous or deleterious substance mentioned must be such as may render such article injurious to health. The word 'may' is here used in its ordinary and usual signification, there being nothing to show the intention of Congress to affix to it any other meaning. In thus describing the offenses, Congress doubtless took into consideration that foods may be used in many ways. It may be consumed when prepared as a food, and as has been spoken of here, by the strong and weak, the old and the young, the well and the sick, and it is intended that if any food, because of any added poisonous or other deleterious ingredient may possibly injure the health of any of them, it shall come within the ban of the statute. . . ."

The jury returned a verdict for the Government.

There is no need at this late date to go into the problems which beset growers and enforcing officials in their attempts to solve the complex problem of poisoning pests without poisoning people. There was much bitterness on the part of growers at one time, but by the middle Thirties most of the technical problems of spray removal had been solved and by 1938 it was only rarely that it became necessary to seize apples or pears because they carried excessive chemical residues.

This, then, was the situation at the time of the passage of the Food, Drug, and Cosmetic Act of 1938. The Secretary of Agriculture had through the years set up advisory and administrative tolerances for residues of the compounds of arsenic, lead and fluorine on apples and pears. The courts had generally supported the views of the enforcing agency as buttressed by the expert testimony of outstanding pharmacologists and physiological chemists. A general acceptance of consumer protective measures in the field of added poisons was to be noted. However, the fact remained that the tolerances so set had no foundation in law but were subject to requirement of proof in each and every case of contest, civil or criminal. Furthermore, the exploratory operations of the enforcing agency in this field necessarily by-passed the conjectural aspects of the potential intake of added poisons from many sources. This became a real problem with the development of data which indicated that the compounds of fluorine might prove to be extremely effective in preventing dental caries in children, and the increasing use of such compounds for this purpose.

The complete history of poisonous chemical residues from 1915 to date was available to the Congress in its consideration of the many measures presented to it from which eventually Senate 5 emerged to become the law of the land on June 25, 1938. The Congress undertook to incorporate in the bill as passed, safeguards consistent with the overall consumer protection features which keynoted the proposed legislation.

Food raw materials are by definition foods. Section 201(f) of the Act states that the term "food" includes articles used for components of any such article. Section 301(a) forbids the introduction or delivery for introduction into interstate commerce of any food that is adulterated or misbranded. Section 301(b) forbids the adulteration or misbranding of any food in interstate commerce. Section 301(c) forbids the receipt in interstate commerce of any food that is adulterated or misbranded and the delivery or proffered delivery thereof for pay or otherwise. Section 402(a)(1) provides that "a food shall be deemed to be adulterated if it

bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health." This means, of course, that a natural poison must be present in such quantities as to constitute a potential menace to the public health. Section 402(a)(2) on the other hand reads: "A food shall be deemed to be adulterated if it bears or contains any added poisonous or added deleterious substance which is unsafe within the meaning of section 406." Section 406(a) reads: "Any poisonous or deleterious substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice shall be deemed to be unsafe for purposes of the application of clause (2) of section 402(a); but when such substance is so required or cannot be so avoided, the Administrator shall promulgate regulations limiting the quantity therein or thereon to such extent as he finds necessary for the protection of public health, and any quantity exceeding the limits so fixed shall also be deemed to be unsafe for purposes of the application of clause (2) of section 402(a). While such a regulation is in effect limiting the quantity of any such substance in the case of any food, such food shall not, by reason of bearing or containing any added amount of such substance, be considered to be adulterated within the meaning of clause (1) of section 402(a). In determining the quantity of such added substance to be tolerated in or on different articles of food the Administrator shall take into account the extent to which the use of such substance is required or cannot be avoided in the production of each such article, and the other ways in which the consumer may be affected by the same or other poisonous or deleterious substances." The legislative history of these sections makes very clear the fact that Congress intended that protection of the public in this field be as nearly as possible absolute.

The Food and Drug Administration was transferred by Presidential Order from the U. S. Department of Agriculture to the Federal Security Agency about two years after the passage of the Food, Drug, and Cosmetic Act of 1938. Under section 406 the Administrator of the Federal Security Agency held hearings and set up a tolerance for fluorine in 1944. In his "Findings of Fact" the Administrator took into consideration the physical and systemic effects of fluorine on humans, particularly in the light of the quantities of fluorine which might be ingested on a total basis in various sections of the United States. The Administrator also took into consideration the possibility that certain drugs might render fluorine more toxic to humans as they demonstrably rendered fluorine more toxic to laboratory animals. He then announced a tolerance of fluorine in terms of the element for apples and pears as "not to exceed 7 milligrams per kilo of each such fruit" or a tolerance of 7 parts per million. This figures out to be 0.049 gr./lb. The regulation establishing this tolerance was invalidated by a decision of the Ninth Circuit Court of Appeals.

During war years it was, of course, physically impossible to hold the hearings necessary to establish tolerances designed fully to meet the aims of Congress as set forth in section 406(a) of the Food, Drug, and Cosmetic Act. After the termination of the war, the Food and Drug Administration began preparations to reopen hearings and set up tolerances under the law for a variety of insecticides which were coming into extensive use. This brought to light a series of serious complications. During the war a large number of new and very potent insecticides had been developed. Little was known about their toxicity, either to the person who applied the sprays or to the consumer who ate the finished product which bore or contained their residues. In some instances we lacked methods for accurate estimation of the residual spray left on or absorbed by the food product. We were unable to conclude whether the residues remained intact, whether they underwent alteration by weathering to become non-toxic or more toxic, whether they could be removed by washing, or whether they were absorbed into the plant structures and could not be removed, or whether indeed they might translocate within the plant and appear in dangerous concentrations in different portions of the plant and its fruit. We just didn't know enough about many of these insecticides to hold hearings and establish safe tolerances.

Two toxicological factors are involved in the consideration of an insecticide. The first is the possibility of acute poisoning; the second is the possibility of chronic poisoning. Since acute poisoning is usually a worker hazard and can be avoided through the use of proper care in the application of sprays and dusts, that problem has not occasioned too much concern from the standpoint of enforcing the Food, Drug, and Cosmetic Act. However, grave concern is properly expressed over the possibilities of chronic poisoning resulting from the long-time consumption of minute amounts of a poison which may eventually build up in the system to produce serious physical disturbances. This type of chronic poisoning may be called cumulative poisoning. It has been recognized for many years that arsenic and lead compounds had this unfortunate propensity. We are just beginning to acquire knowledge, however, about the cumulative toxicity of a few of the newer insecticides. I will not spend time today on interim reports of toxicological studies which have been conducted by the Food and Drug Administration for the past several years in seeking the answers to the questions raised above, but will devote this time to a brief discussion of our latest information and present views on several of the newer insecticides which have come to be rather widely used.

DDT

DDT proved to be a tremendously useful pesticide in World War II. Its use undoubtedly saved the lives of many members of the Armed Forces who otherwise would have succumbed to typhus and malaria. There was no question even at that time but that DDT was poisonous. Work in the laboratories of the Public Health Service and the Food and Drug Administration, done at the request of the military authorities, proved that, but the use of DDT was a reasonably calculated military risk. This was based on the premise that the

use of DDT by the troops, while in fairly heavy exposure, would not be long continued: the cumulative poisoning hazard would be low. Since the war DDT has assumed prime rank as an extremely effective insecticide. With its expanded use in many fields there has developed, of course, an increasing danger of exposure by the general public to small but frequent quantities of this product from many sources and over long periods of time. This brings the hazard of cumulative effect to the front. In discussing DDT at a recent meeting of the American Chemical Society, Dr. A. J. Lehman, Chief of the Division of Pharmacology of the Food and Drug Administration, stated: "Further studies on the accumulation of DDT in body fat reveals that this insecticide is stored in the fat of rats when fed at a level of 1 p.p.m. in the diet. There is a progressive rise in storage with time, reaching a maximum after 23 weeks. This time period is independent of the level of intake within the range of 1 to 10 p.p.m. Old and young rats accumulate DDT at the same rate. The rate of disappearance of DDT from the animal body is slow. One-fourth of the total still remains after the animals have been placed on a DDT-free diet for three months. Evidence of liver injury has been observed in rats consuming a diet containing 5 p.p.m. from four to six months."

As yet we do not know how serious this hazard is in terms of human damage. We do know that female dogs exposed to the effects of DDT secrete it in milk and that mother rats fed 50 p.p.m. or more produce smaller weanling rats with fewer survivals to a litter than control animals. We know also from experiments of the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture that if dairy cows are fed ensilage bearing DDT, if dairy cows are sprayed with DDT, or if DDT is used in dairy barns, the milk from those cattle will contain DDT. Prior to the reporting of these experiments it was the view of the Food and Drug Administration that with proper precautions to protect the food from contamination, DDT was a useful and safe insecticide to be used in connection with food production of every type. Following these reports, however, there was only one answer. Milk is a universal and vital food. It is the principal food of babies and an important food of children and a widely used food through adult life. Its purity must be safeguarded in every possible way. We will not and cannot set up a tolerance for DDT in milk. Fortunately, the Bureau of Entomology and Plant Quarantine has been able to suggest alternatives and less objectionable substitutes in place of DDT within the dairy industry. In announcing what he considered to be a safe figure for DDT residues, Dr. Lehman stated that DDT should be present in concentrations of less than 1 p.p.m. if all of the food carries residual DDT and not more than 5 p.p.m. in any single item.

Benzene Hexachloride

The Food and Drug Administration has been testing benzene hexachloride with particular emphasis on the pharmacological action of its isomers for nearly two years. The last report on these experiments at the end of the 20th month indicates the order of toxicity is as follows, using the Alpha isomer as the base: Alpha, 1; Beta, 2; Gamma, 1; Delta, 0.5. None of the isomers appear to affect growth in laboratory animals at the 100 p.p.m. level of feeding although slight histological changes in the liver have been noted. Beta produced questionable liver damage at the 10 p.p.m. level of feeding. Distribution studies on the four isomers showed that fat storage of Alpha has a ratio of about 2 to 1 over the concentration in the whole diet. Beta has a ratio of about 10 to 1, while Gamma and Delta are deposited in the tissues at about the same concentration as contained in the diet or at a ratio of 1 to 1. Alpha disappears fairly rapidly, approaching zero in about a month. Beta on the other hand is mobilized very slowly and does not disappear entirely until 3 to 6 months have elapsed. Gamma and Delta disappear completely within two weeks. Technical grades of benzene hexachloride are skin irritants, the damage being almost in direct proportion to the amount of Gamma isomer present. Dermal application can produce toxic effects and the toxicity of the Gamma isomer on repeated exposure increases many fold. Generally benzene hexachloride isomers give a pathological picture not unlike that caused by DDT with liver damage as the characteristic finding. According to Dr. Lehman quantities of benzene hexachloride present on food at time of use should not exceed 1 p.p.m. if all the food contains the chemical, and not over 5 p.p.m. in any single item.

Parathion

Parathion is dangerous to the operator because of its acute toxicity, but since it rapidly breaks down in the body and lacks cumulative properties, studies to date indicate that an amount up to about 2 p.p.m. as a residue at harvest time probably is safe from the standpoint of the consumer. This figure, of course, is subject to change as more information becomes available and applies only to a single item of food. Experimental applications of Parathion on apples and pears, when used in concentration up to 2 lbs. of Parathion (25%) per 100 gallons with four sprays applied, the last being applied not later than a month before harvest, resulted in residues of Parathion at time of picking of only a small fraction of 1 p.p.m. The applications actually used in these experiments would give an initial load well above that resulting from commonly recommended spray schedules. The eating of pears or apples freshly sprayed with Parathion would result in acute poisoning, but with strict adherence to spray schedules and spraying not later than a month before harvest, residues should not present a problem in the case of these two fruits. This does not necessarily follow in the case of citrus fruits where the peel itself may constitute an article of food.

Tetraethyl Pyrophosphate

From the standpoint of acute toxicity Tetraethyl Pyrophosphate is one of the most fearsome of the new insecticides. An organic phosphate like Parathion, it penetrates the skin rapidly. As little as 0.3 of a gram on a daily exposure basis is dangerous to man. Inter-

nally the organic phosphates are estimated to be from three to five times as toxic as nicotine, and 12 to 20 milligrams or one-fifth to one-third grains may be liable to cause fatality. As little as one small drop of the concentrate splashed into the eye may be fatal. From the standpoint of chronic toxicity, Tetraethyl Pyrophosphate does not present a grave problem since it decomposes and the known products of decomposition are not considered to be hazardous.

Others

Other new insecticidal agents, including the analogs of DDT (Methyoxchlor and TDE), Chlordane, Toxaphene, Piperonyl Butoxide, and insecticide activators are also being actively studied in the pharmacological laboratories of the Food and Drug Administration. Preliminary work indicates that in no case should the concentration of these agents in a single food exceed 10 p.p.m., and in Chlordane, Toxaphene, and TDE not over 5 p.p.m.

From this brief recital it is obvious that "bug killing" procedures in this country have undergone fundamental and radical changes within the last decade. Well known poisons have been largely supplanted by new highly effective and extremely toxic agents. The toxicities of these various agents, acute and chronic, are still being studied. Their potentialities for damage through cumulative poisoning over a period of years necessarily are largely unexplored. This entire field calls for the most painstaking and honestly reported fundamental research which will let the "chips fall where they may." That is an obligation which, in my judgment, should be of first concern to the manufacturers and primary distributors of these insecticidal agents.

In a recent talk before the American Association of Economic Entomology, Deputy Commissioner of Food and Drugs C. W. Crawford, stated: "I cannot stress too strongly the necessity for a thoroughgoing investigation of all phases of toxicity of every pesticide intended for use in connection with food production where there is any likelihood that even the merest trace may remain in or on the food at the time of consumption. To produce and use any product before this basic research work has been done is to invite disaster in terms of human suffering and life. To commercialize a poison before its potentialities are known is simply to use the public as guinea pigs." Mr. Crawford went on to say: "As an organization vitally interested in this problem the Food and Drug Administration's Division of Pharmacology will be glad to discuss with anyone who is interested, the scope and kind of investigations it believes through its experience, should be carried out before any products are exploited."

These same conditions have imposed a heavy responsibility on those who recommend the use of these insecticidal agents and who undertake to work up spray schedules. Their approach must be conservative and the harmful potentialities of these products must never be overlooked or ignored. Those who use these insecticides likewise assume a heavy burden of responsibility to follow strictly such schedules as represent the mature judgment of the agencies which prepare them, to safeguard adequately the workers who apply these agents in the form of sprays or dusts, to prevent accidental contamination of neighboring areas, and to make such tests as are necessary to insure that products as harvested for market do not carry dangerous residues. The manufacturer who utilizes as raw materials foods which have been subjected to treatment with one or several of the newer insecticides must likewise accept responsibility for insuring that such raw materials do not carry dangerous loads of added poisons. He must ever bear in mind the possibility of the development of dangerous concentrations in by-product utilization. Each must pay at his level the price of concentrated production and the complexities of a highly developed economy.

On the other hand, the agency enforcing the Food, Drug, and Cosmetic Act has a clear mandate from the Congress. It must, under the terms of the FD&C Act, prevent interstate movement to consumers of products which may alone or in conjunction with the ingestion of other products similarly treated create a health hazard, actual or as defined by statute. This multitude of problems will in time be solved as have many which have come before. The technological research activities of the Food and Drug Administration's Division of Pharmacology have now progressed to the point where we feel that we can proceed to the establishment of tolerances as provided in section 406 for those insecticidal agents which are required in the production of certain staple fruits which have wide use in the American dietary. Field offices of the Food and Drug Administration are now collecting data looking toward the adoption of such tolerances, after appropriate hearings, for certain types of spray residue on apples and pears. It is possible that other fruits and some vegetables may be included if the facts warrant. At the present time it is the thinking of the Food and Drug Administration that tolerances should be proposed and established for the compounds of arsenic, lead and fluorine and for DDT and Parathion on apples and pears. It may be desirable after analysis of the country-wide situation to extend these proposals. As soon as hearing dates and subject matter are finally decided, wide distribution will be made of this information to the end that appropriately interested growers, distributors, processors, manufacturers and consumers may have an opportunity to present their views as to what constitutes an appropriate tolerance under the terms of the law for each such agent on specific fruits and/or vegetables. Meantime, the Food and Drug Administration is continuing to operate on the basis of informal tolerances for lead and arsenic on apples and pears of 0.05 gr./lb. and 0.025 gr./lb. respectively. Growers have been informed that in the judgment of the Food and Drug Administration, DDT residues on apples and pears should be kept below 7 p.p.m. or 0.049 gr./lb.

Pending the setting of tolerances as provided by the statute, the Food and Drug Administration urges conservatism and great caution in the distribution, promotion and utilization of the powerful weapons which have been added to this field within the past decade.

Experimental Bulk Fermentation of California Green Olives

R. N. BALL, ED. VAN DELLEN AND J. B. JAQUITH
Pacific Olive Company, Visalia, California

REESE H. VAUGHN, JOSEPH TABACHNICK AND G. T. WEDDING
Division of Food Technology, University of California

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Studies concerning the relative merits of the conventional fermentation of 275 pounds of olives in 50 gallon barrels as compared with fermentation in bulk in redwood tanks which contain about 15,000 pounds of olives indicate that there is no significant gross chemical or bacteriological difference between the two processes. The bulk fermentation, if used under the direct supervision of trained personnel, is economical of labor and time and insures a greater uniformity of product than does the conventional barrel process.

I. Introduction

The extensive commercial-scale fermentation of green Spanish-type olives in California is a comparatively recent development although, according to Wetmore *(9)*, successful attempts were made as early as 1889 and directions for pickling green olives had been written as early as 1880 by Cooper *(2)*. However, for some reason, by 1900 the California ripe olive had become established as a main product of the industry whereas the green fermented olive was considered more or less indifferently until about 1938.

Adaptation of the Spanish process, apparently first described by Gracey in 1918 *(5)*, by Cruess in 1924 *(3)* and later by Bull in 1936 *(1)*, to California conditions was slow. The first commercial-scale experiments were reported in 1930 by Cruess *(4)*, but it took conscientious effort on the part of the entire industry to adjust the fermentation to the different conditions which prevail throughout the state. Now, largely through experience gained since 1935, any group in the industry can produce successfully green fermented olives.

One of the chief obstacles to commercial-scale fermention of green olives in California has been the high cost of production as compared with the cost in Spain. Much of the hand labor used in Spain has been eliminated in the California industry by the use of such time- and labor-saving devices as size-grading machines, mechanical barrel hoop tighteners, olive pumping and fluming systems and lift trucks. To save further time and labor it seemed logical to attempt to eliminate the use of the conventional 50 gallon size barrel fermentations. Some precedent for this attempt already was established. The Spanish regularly use large casks of about 170 gallons capacity for their olive fermentations. Picklers sometimes use large tanks for their dill pickle fermentations. Accordingly, a study of the fermentation of olives in bulk in tanks was undertaken.

II. The Bulk Process

The basic steps in the production of fermented green olives in California are essentially the same as those used in Spain, although, as already intimated, certain adaptations of the process have been found to be advantageous.

Conventional method for production of California green olives. The process requires that only freshly harvested, mature, green to straw yellow olives be used. These are placed in redwood or reinforced concrete tanks and treated with lye solution. The sodium hydroxide is required to hydrolyze the bitter glucoside, *oleuropein*, because too much residual bitterness is objectionable in the fermented fruit. The caustic solutions vary between 1.25 and 2.00 percent sodium hydroxide. The lye is allowed to penetrate from one-half to three-fourths of the way to the pits of all of the olives treated. The excess caustic is then removed by leaching the fruit with water.

After removal of all but traces of the sodium hydroxide the olives are placed in oak barrels of about 50 gallons capacity. Each barrel is filled with about 275 pounds of olives and enough salt brine to completely cover the fruit. The filled and brined barrel then is rolled into the fermentation yard.

The fermentation may be completed in from three weeks to as long as one year, depending upon the amount of technical control. It is possible and also practical to complete the fermentation in three weeks to one month under commercial conditions. To accomplish this it is necessary to integrate carefully control measures which require the maintenance of a brine temperature of 75° F. (24° C.); the adjustment of the salt concentration of the brine to 5 to 7 percent (grams NaCl per 100 ml. of brine); the presence of desirable lactic acid bacteria in the brines; and the addition of supplementary sugar (glucose or sucrose) to the brines to insure that an acidity of 0.8 to 1.0 percent lactic acid (total acidity calculated as grams lactic acid per 100 ml.) is produced.[a]

Fermentation in bulk. Analysis of the conventional process indicated that the greatest saving of time and labor would accrue by lye-treating and fermenting the olives in the same large container. Hence, experiments conducted over a ten-year period have been designed to test this assumption.

Containers investigated have included open and closed tanks of redwood and reinforced concrete. Results with concrete tanks have not been completely satisfactory. A coating is required to protect the concrete from the corrosive action of the lactic acid formed during the fermentation. Furthermore, once the reinforced concrete tank is constructed, it is completely

[a] The Sevillano, Manzanillo and Barouni varieties, commonly fermented in California, react differently in processing and fermentation. For a more detailed discussion of these processing and fermentation variations, consult Cruess *(4)*; Vaughn *(6)*; and Vaughn et al. *(7, 8)*.

immobile. Redwood tanks do not have these disadvantages but, unlike the concrete ones, must not be allowed to dry out when not in use. Despite this latter disadvantage, redwood tanks are preferred.

The tanks may be located out of doors or in specially constructed incubation rooms. If located outside, provisions must be made for heating the tanks so that the brines may be incubated at a controlled temperature. One of the simplest methods to provide incubation for tanks out of doors is to elevate them, enclose the bottoms and bleed live steam into the enclosed space under the bottoms. If the tanks are located indoors, the incubation room is maintained at a temperature which will insure that the brines will be incubated at a desirable temperature. Temperatures from about 75 to 85° F. (24 to 29.5° C.) are used.

The only significant difference between the conventional and the tank or bulk fermentation is the larger container used in the latter method. This container, usually a redwood tank made with staves 4 feet long and a diameter of 14 feet, serves both for lye-treatment and fermentation of the olives. Thus the small cooperage and rehandling of fruit required in the conventional process are eliminated. Otherwise, the same careful control is required in selection of fruit, lye-treatment and fermentation of the olives.

In the bulk process the tank is filled to within about 12 to 18 inches of the top with freshly harvested olives of the proper maturity. A slatted cover (Figure 1) is fitted over the olives and securely anchored in place. The cover keeps the olives submerged during lye-treatment, washing and fermentation. It also acts as a sub-top when the tank is sealed during fermentation. The fruit is then covered with lye solution of the desired strength and lye-treatment is allowed to proceed. After the sodium hydroxide has penetrated to the desired depth in the flesh of the olives, leaching is started. After several changes of water (usually at least four), the last water added is left to cover the olives, and brine is formed by addition of salt until the concentration in the liquid is about 6 to 7 percent (grams NaCl per 100 ml. of brine). When fermentation starts it is controlled carefully and kept going by addition of sugar until at completion from 0.8 to 1.0 percent lactic has been formed.

FIG. 1. Showing the bulk fermentation tank, the slatted cover and conventional 50 gallon barrels. The slatted cover is constructed in two half-circles for ease of handling.

When the fermentation is well under way and the salt concentration in the brine has stabilized, the tank is closed. This is done by flooding the brine and slatted cover with a layer of melted paraffin or microcrystalline wax. On solidification either of these materials forms a tight cover which seals the fermentation off from the air. In this way the fermentation is protected from the activity of oxidative yeasts which otherwise might abound and waste both sugar and lactic acid.

Because the fermentation is kept at a uniform temperature by incubation, there is no marked diurnal expansion and contraction of the brine experienced when barrels of olives are kept out of doors for fermentation.

Supplementary sugar is required to insure the production of sufficient lactic acid. Crystalline corn sugar (glucose) is commonly used. The sugar is added at the rate of 1 pound per 50 gallons. A total of 2 to 3 pounds per 50 gallons is usually required to decrease the pH value to at least 3.8, which is considered a safe level. At this pH value the total acidity calculated as grams lactic acid per 100 ml. of brine will vary from 0.6 to 0.8 percent.

TABLE 1

Comparison of gross chemical and bacteriological changes in bulk and barrel fermentation of Manzanillo olives

Bulk Fermentation 15,000 pounds						Barrel Fermentation 275 pounds					
Time in days	Brine temp. ° F.	Brine temp. ° C.	pH	Acidity, grams lactic per 100 ml. of brine	Total lactic acid bacteria per ml. of brine [b]	Time in days	Brine temp. ° F.	Brine temp. ° C.	pH	Acidity, grams lactic per 100 ml. of brine	Total lactic acid bacteria per ml. of brine [b]
0	62	16.5	11.4	0	0	0	57	14	10.3	0	0
5	64	18	10.0	0	less than 100	7	62	16.5	4.9	0.144	1,150,000
12	60	15.5	6.5	0.028	96,000	14	67	19.5	4.8	0.293	19,300,000
19	64	18	4.9	0.162	2,610,000	21	68	20	4.3	0.527	143,000,000
25	69	20.5	4.6	0.234	22,800,000	28	62	16.5	4.4	0.502	60,000,000
31	66	19	4.0	0.450	21,050,000	35	60	15.5	4.3	0.521	36,000,000
40	78	25.5	4.0	0.468	11,000,000	43	57	14	4.4	0.530	58,000,000
48	73	23	3.8 [c]	0.621	51,100,000	51	55	13	4.1	0.540	20,000,000
						58	58	14.5	4.0	0.520	16,000,000
						65	56	13	4.0	0.535	11,000,000
						72	42	5.5	3.8 [c]	0.603	25,500,000

[b] Counts include only lactic acid bacteria able to grow on yeast infusion glucose agar at pH 5 to 5.5. Yeasts and coliform bacteria which, if present, may also grow were carefully differentiated by visual and microscopic means. The populations only show trends.

[c] The fermentations are commonly directed until the pH value decreases to 3.8 or less. The olives must be free of sugar and cured before packing.

It has been found that the gross chemical and bacteriological changes which occur in the bulk fermentation parallel almost exactly those observed in carefully controlled barrel fermentations, as is shown in Table 1. However, the bulk fermentations are incubated once the prevailing out-door temperatures become unfavorable. For this reason, acid production in the bulk fermentation of olives is finished in a relatively short time as compared with the conventional barrel fermentation which is not incubated. Figure 2 summarizes the trends in acid production for bulk fermentations and unincubated conventional barrel fermentations.

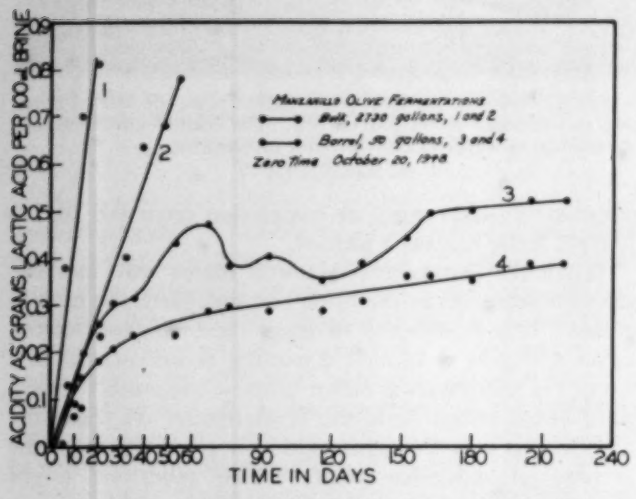

FIG. 2. Showing the difference in acid production in controlled bulk fermentations compared with conventional barrel fermentations.

III. General Advantages of the Bulk Process

There is a decided saving of labor and time which results from the use of the bulk process for fermentation of green olives. There also is a saving in cooperage and coopering which results under conditions where the processor packs the fermented olives in glass.

With the bulk process it is possible to ferment up to 15,000 pounds of olives per tank. The saving in cooperage is obvious: the tank containing 15,000 pounds of olives is equivalent to about 16 Spanish casks or almost 55 California barrels.[4]

The labor saving advantages are evident in all phases of the process: no rehandling of olives is required after lye-treatment; no daily brining of barrels is necessary; and, above all, labor is saved in control of the salt, sugar and temperature requirements of the fermentation.

The bulk fermentation is especially time saving for control personnel. As already stressed, it is desirable to follow the fermentation of green olives closely in order to direct the course of the fermentation. With the tank process it is easy to conduct the required chemical and organoleptic tests each day, if necessary, and not neglect other duties. On the other hand, such close checking of barrel fermentations with the required control tests is impossible.

[4] Calculated on the basis of one U. S. gallon of olives weighing 5.5 pounds; the Spanish cask containing 170 U. S. gallons; and the California barrel containing 50 U. S. gallons.

It is also significant that the bulk fermentation allows for more rapid handling of the fresh fruit. Furthermore, there is less undesirable darkening of the lye-treated olives which is caused when they are exposed too long to the air before fermentation. Too, there is less breaking and crushing of fruit because the barreling operation is eliminated. Finally, there is greater uniformity of product with olives fermented in bulk as contrasted with the variations experienced from barrel to barrel with the conventional method.

These advantages which accrue from the use of the bulk fermentation process may be completely vitiated unless competent, trained personnel is directly in charge of all operations. There is grave danger of serious economic loss unless the bulk fermentation is subjected to rigid control. The losses which might result from undetected microbial spoilage or leaking tanks are obvious.

An inherent disadvantage of the bulk fermentation is the problem of closing the tanks. Open tanks are used to facilitate control of lye-treatment. Although it is not difficult to seat the slatted cover and flood it with melted paraffin, it is difficult to keep the tank properly sealed. Constant attention is required to close fissures which result from accumulation of fermentation gas. If the tank is located inside, the growth of mold must be prevented. If the tank is located outside, rain as well as gas causes disruption of the seal.

IV. Summary

Studies have been made concerning the relative merits of the conventional fermentation of 275 pounds of olives in 50 gallon barrels as compared with fermentation in bulk in redwood tanks which contain about 15,000 pounds of olives. The results obtained indicated that there is no significant gross chemical or bacteriological difference between the two processes.

The bulk fermentation process is economical of labor and time only if it is under the direct supervision of trained personnel; otherwise it is a potentially hazardous method because of the danger of losses which might result from acute microbial spoilage of the olives.

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Factors Affecting the Keeping Quality of Bacon[a]

O. G. HANKINS, WILLIAM L. SULZBACHER, W. R. KAUFFMAN, AND MARTHA E. MAYO[b, c]

Bureau of Animal Industry, Agricultural Research, Administration, U. S. Department of Agriculture, Beltsville, Maryland

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The primary purpose of this study was to develop a means for improving the keeping quality of mildly cured, sliced, vacuum-packed bacon when stored at relatively high temperatures. The investigation comprised a determination of the amount of salt generally tolerable in "mild" bacon, as well as a study of the moisture: salt ratio of such bacon and its effect on the storage life and bacterial flora of the bacon, together with a study of processing methods for varying this ratio. The microorganisms responsible for spoilage of mild, vacuum-packed bacon were isolated, the mechanism by which they produced swollen cans determined, and a possible bacon sterilizing agent was investigated.

This study was undertaken in an attempt to improve the keeping quality of canned bacon prepared for overseas use by the U. S. Army. War-time experience demonstrated that the sliced, vacuum-packed "Overseas" type of bacon, as well as the 10-in-1 type which was a later development, would keep satisfactorily for relatively long periods of time at unfavorable temperatures. Both of these products, however, were subjected to a great deal of criticism and were widely unacceptable for Army use. This was due to excessive saltiness in both instances,—more so in the former than the latter, and also to poor texture resulting from a pasteurizing treatment in the case of the 10-in-1 product.

As a result of this situation, considerable interest developed in the possible use of the familiar, mildly-cured, commercial type of bacon. Tests indicated, however, that when sliced, vacuum-packed, and stored at 100° F. (37.8° C.) it became sour in 3 months' time or less. It was thus evident that if bacon of this type is to have a place in Army rations, requiring long-distance shipping and long-time storage under unfavorable temperature conditions, research on the problem was required.

Objectives of the Study

The objectives of the study reported here, as originally outlined, were (1) to identify the microorganism or microorganisms that cause spoilage in mildly-cured, sliced, vacuum-packed bacon during storage at relatively high temperature and (2) to determine the effect of the moisture: salt ratio in such bacon on the keeping quality of the product. Added later was the objective to study the usefulness of ethylene oxide as a sterilizing agent and as a possible means of preventing the spoilage of this type of bacon.

Materials Used

Complying with a request from the Bureau, the Office of the Quartermaster General arranged for the processing by a commercial packer of three separate lots of typical, mildly-cured, sliced, vacuum-packed bacon. This bacon was used for initial storage experiments and provided a useful standard for comparison with later experimental lots of bacon produced at Beltsville.

The bacon used in later storage studies was produced, processed and canned at Beltsville. It came from hogs weighing approximately 225 pounds and of uniform feeding, which had been produced in record-of-performance studies. Whenever possible, curing was begun a few days after slaughter. When this was not feasible the bacon was held at 0° F. (—17.8° C.) until ready for cure and then thawed at 36° F. (2.2° C.) before using. In curing, fine common salt, Federal Specification No. SS-S-31 Type A, commercial brown sugar, and C. P. potassium nitrate were used.

Methods Employed

Curing and smoking. The sides of bacon to be cured were placed in shallow pans and uniformly covered with 2.85 percent of their weight of a curing mixture composed of 1 pound of salt, 0.1 pound of sugar and 0.04 pound of potassium nitrate. The sides were left in cure for 14 days at 38° F. (3.3° C.) and then hung on bacon hooks and washed well with a stream of hot water from a hose. After drying, the bacon was smoked with hardwood smoke in a steam-heated smokehouse. The temperature of the house was held at about 135° to 140° F. (57° to 60° C.) until the internal temperature of the bacon reached 120° F. (49° C.). This usually occurred in about 4 to 6 hours. The temperature of the house was then allowed to drop to 110° to 120° F. and smoking continued for a total of 24 hours. This procedure was adopted as the standard curing and smoking method for the project after some preliminary work was done.

Heating. Two methods of heating bacon after smoking were employed—(1) in the smokehouse and (2) in a dehydrator of cabinet type through which air was circulated. Both methods involved a temperature of about 120° F. (49° C.)

Slicing. Bacon was sliced with an electrically driven machine and the strips were laid shingle fashion on vegetable parchment paper. Samples were taken for chemical analysis at the time of slicing. The sampling was done in such a manner that the bacon used for analysis was strictly representative of that which was canned.

Inoculating. Immediately after slicing, the bacon was sprayed by means of an atomizer with a suspension of known spoilage organisms or organisms being tested for ability to cause spoilage.

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[a] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned Number 267 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

[b] Transferred to U. S. Bureau of Standards.

[c] Acknowledgment is made to Eleanor E. Weinstein and Deborah S. Levin for assistance in the work.

Canning. The strip was then rolled into cylindrical form and inserted into a round metal can, size 307 x 305 or 307 x 400, with soldered side seam and compound-lined, double-seamed ends. The cans were then sealed under vacuum.

All of the Beltsville-produced bacon was stored at 37° C. (98.6° F.) after canning. Of the three lots of commercially-produced bacon received at Beltsville through arrangements made by the Office of the Quartermaster General, 45 cans of each lot were stored at 37° C., one can at 1.1° C. (34° F.) and 24 cans at room temperature.

Bacteriological methods. Before a can of bacon was opened for counts or isolation of microorganisms, the outside of the can was thoroughly washed with soap and water and then allowed to stand for several minutes in a saturated solution of mercuric chloride. The can was then rinsed with sterile water and opened with a sterile can opener. Several slices of bacon were removed from different parts of the roll with sterile forceps and placed in a previously weighed, sterile Petri dish. The Petri dish was again weighed and the weight of the bacon computed. Between 10 and 12 grams of bacon were used. The bacon was next placed in a sterile Waring blendor jar containing 100 ml. of physiological saline and blended. Various dilutions were made from the suspensions of bacon in saline and plates were poured, either veal infusion medium (Difco) with the addition of 1.5 percent agar, or pork heart agar being used. Duplicate sets of plates were poured. One set was incubated aerobically and one set anaerobically in a Brewer or Spaulding anaerobic jar. The plates were incubated at 37° C. for 24 to 48 hours and the colonies were counted.

A portion of the suspension of bacon was inoculated into thioglycollate medium (Difco) and tubes of Jensen's spiced ham medium *(6)* by means of a Pasteur pipette. After 24 to 48 hours of incubation all tubes showing gas production were streaked on veal infusion agar and isolated colonies were later picked. Thioglycollate and Jensen's medium were inoculated with pure cultures of the organisms isolated and the tubes were observed for evidence of gas production. A modification of Jensen's spiced ham medium was employed in which bacon was substituted for the ham. Results were the same with either medium. All tubes and plates were incubated at 37° C. The organisms which were isolated from cans of spoiled bacon were identified on the basis of morphology and biochemical reactions, using the usual bacteriological media.

A number of the organisms isolated were tested for CO_2 production with the use of home-made fermentation tubes of the Eldredge *(4)* type with standard barium hydroxide as an absorbent. For this phase of the work Difco nutrient broth with the addition of 0.3 percent yeast extract and 1 percent dextrose was used.

Chemcial determinations. Determinations of moisture, ether extract, and total nitrogen were made according to the Methods of Analysis of the Association of Official Agricultural Chemists *(1)*. In the Sorensen amino nitrogen determination, the above book of methods was also followed except that all coagulable proteins were removed from the solutions before titrations were made. Sodium chloride was determined by an adaptation of the Dyer *(3)* method. In determining free fatty acids a rapid extraction procedure was used. Small quantities of bacon were mixed with small amounts of anhydrous sodium sulfate and purified sand and the mixture placed in an Erlenmeyer flask. Enough Skelly "F" was then added to cover the sample. A little heat was applied by means of a steam bath and, after shaking, the extract was decanted through a filter into a weighed flask. This procedure was repeated several times in order to obtain fairly complete extraction. The extract was evaporated to dryness on a steam bath and the flask then placed in a vacuum oven at 100° C. for several hours. A final weighing gave the weight of the extracted fat. This was then titrated according to the method described by Jamieson *(5)*.

Results

Preliminary tests. In order to establish a standard curing procedure for the production of mild bacon to be used for the experimental canning and storage in this study, several preliminary tests were run to determine the rate of salt absorption by the "green" bellies and the optimum time required for curing. The curing mixture and procedure were based on Tentative Specifications, Q. C. D. No. 155 A, of the Quartermaster Corps.

It was originally thought that the salt content of the product could be effectively controlled by removing the sides from cure after an adequate period of time had elapsed. However, the first tests demonstrated that the salt was absorbed so rapidly that only about a week in cure was required to attain a relatively high salt concentration on the average, but that considerable variation occurred. It was then decided that a more logical and precise method would be to apply only a sufficient amount of curing mixture to provide the desired concentration in the finished product and to leave the bacon in cure long enough to absorb all the salt applied.

Figure 1 shows the results of four preliminary curing experiments. Lots A and B were cured by applying

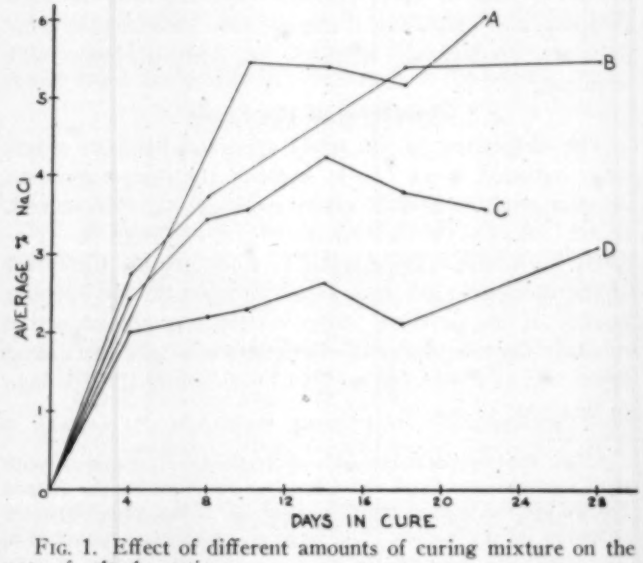

FIG. 1. Effect of different amounts of curing mixture on the rate of salt absorption.

6 pounds of curing mixture per 100 pounds of meat and adding 3 pounds per 100 pounds of meat on the 16th day in cure. The curing mixture was composed of 90 percent NaCl, 9 percent sugar, and 1 percent KNO_3. Lot C was cured with only the original 6 pounds of

curing mixture and Lot D was cured with only 2.36 pounds per 100 pounds of meat. In sampling the bacon at successive intervals during curing, pieces were cut alternately from the anterior and posterior ends of paired sides each time, and the salt content recorded was the average of these two pieces. This method was believed to represent the best available means for obtaining comparable samples, although the irregularity of the curves in Figures 1, 2 and 3 indicates that it was not perfect.

It is made clear by Figure 1 that there was a very rapid absorption of salt by all lots during the first few days. In Lots A and B this continued for from 10 to 14 days and in Lot C up to the eighth day. A clear-cut break in the curve for Lot D appears at the 4-day stage. It is noteworthy that the final salt percentage attained by Lots A and B were approximately 5.5 to 6, and by Lot C about 3.7. For Lot D the estimated maximum was approximately 3.2 percent. It is apparent that some of the salt, in Lots A, B and C, was not absorbed. However, in Lot D, it would appear that all of the salt applied was absorbed and the salt content of the bacon was increased to the estimated maximum of 3.2 percent as a consequence of the moisture losses during the curing and smoking operations.

To throw further light on the problem relating to salt absorption and moreover distribution, additional lots of bacon were cured by the 90-9-1 mixture described above. The results of these experiments are shown in Figures 2 and 3. For the lot described by Figure 2, a curing mixture equal to 4 percent of the weight of the bacon was used and for Figure 3, 3 percent was employed. The sides were sampled as before except that salt analyses were run on the outer portion of the bacon, the inner portion next to the skin, largely fat, and the bacon as a whole. It will be noticed from Figures 2 and 3 that the bacon as a whole had absorbed the maximum amount of salt by the 14th day, assuming that the slight rise in Figure 3 from the 18th to the 21st day was due to errors inherent in sampling. Similarly, the low salt Lots C and D in Figure 1, when the variation of sampling is considered, did not increase greatly in salt content following the 14th day. In all cases the rapid salt absorption during the first few days in cure is noteworthy.

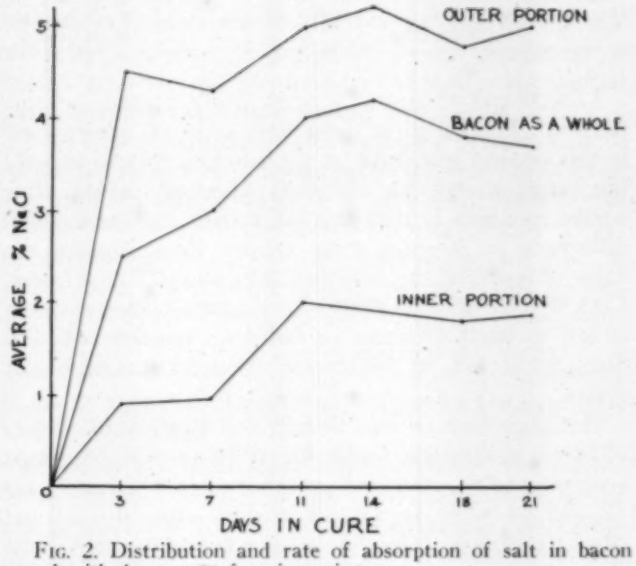

FIG. 2. Distribution and rate of absorption of salt in bacon cured with 4 percent of curing mixture.

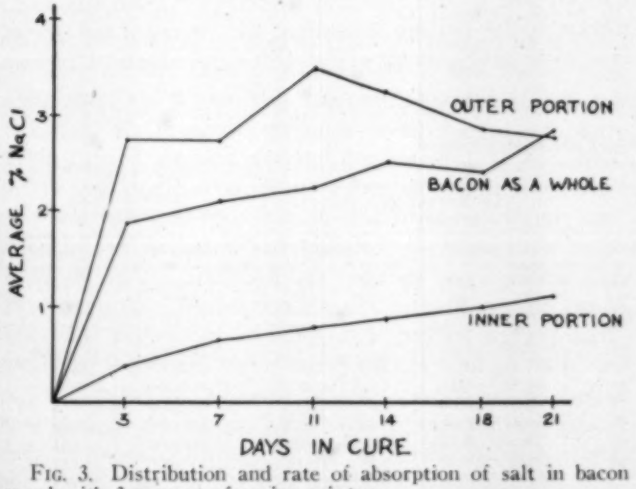

FIG. 3. Distribution and rate of absorption of salt in bacon cured with 3 percent of curing mixture.

During this preliminary curing work samples of bacon varying in salt content were fried and tasted by a panel of judges. The preferences expressed are shown in Table 1. Although there was considerable variation in the results, it was concluded from them that, to be acceptably mild, bacon should contain not more than 3.0 percent of NaCl prior to cooking.

TABLE 1

Opinions of judges testing lots of bacon varying in salt content

NaCl	Percent of judges who scored samples		
	Too salty	Acceptable	Not salty enough
(percent)			
1.45*	0.0	47.0	53.0
1.02*	0.0	45.5	54.5
1.26*	0.0	47.0	53.0
1.60	19.0	53.0	28.0
1.61	0.0	55.5	44.5
1.71	0.0	44.5	55.5
2.00	19.0	66.7	14.0
2.33	25.0	50.0	25.0
2.55	15.4	83.9	0.7
2.58	14.3	71.4	14.3
2.58	19.0	81.0	0.0
3.04	37.0	63.0	0.0
3.11	8.8	56.5	34.7
3.17	0.0	72.7	27.3
3.32	33.3	66.6	0.0
3.38	38.5	60.8	0.7
3.47	31.0	63.0	6.0

* Three lots of commercial bacon.

From these preliminary experiments the method of curing given under "Methods Employed," above, was chosen as the standard method for the production of mild bacon for the purposes of the project. The curing mixture was compounded as indicated (1 pound NaCl, 0.1 pound sugar, and .04 pound KNO_3) for convenience in weighing and the value, 2.85 percent of the green weight, was chosen on the basis of previous shrinkage records of bacon being smoked at Beltsville, as likely to give 3 percent in the finished product. An analysis of a typical lot of bacon cured in this way showed values of 2.74 percent NaCl, 22.38 percent moisture, 3.15 percent ash, 11.86 percent protein, 62.76 percent ether extract, and an average moisture: salt ratio of 8.2:1.

Moisture: salt ratio in relation to keeping quality of bacon. The good keeping qualities of the sliced, vacuum-packed "Overseas" type of bacon indicated that a high salt content was valuable in conferring a long shelf life on the product. Some way was needed to take advan-

tage of the bacteriostatic qualities of the salt without rendering the bacon excessively salty when eaten. It seemed logical that if the moisture content could be significantly reduced a mild commercial type of cure might be just as valuable in preserving the product as a high salt cure would be in preserving a more moist product. The first method tried for reducing the moisture content of the bacon was to hang the sides in a smokehouse after the normal smoking period was completed. During this additional period the house was heated by steam coils, without smoke, and maintained at 120° F. (49° C.). Five lots of bacon were prepared to be used for a comparison of the effect of moisture: salt ratio on keeping time. One lot was given a mild cure (using only 1.5 percent of the standard curing mixture) and smoked in a house to which moisture was added as steam. The second lot was cured by using 2 percent of the curing mixture and smoked under the same conditions as the previous lot. Lot 3 was cured and smoked under the standard conditions used when 3 percent of the curing mixture was employed. Lots 4 and 5 were cured and smoked by standard methods, but Lot 4 was heated for 3 days and Lot 5 for 6 days after smoking. Since the analyses of these 5 lots of bacon did not show great disparity in salt and moisture values, the experiment was repeated and only the high, standard, and low moisture:salt ratios were sought. These were called Lots 1A, 3A, and 5A and were processed identically with Lots 1, 3 and 5 described above. All the bacon was sliced, inoculated, and canned with the use of the standard procedures described. The results obtained in this experiment are shown in Table 2, together with the corresponding data for the three lots of commercially processed bacon obtained from the Quartermaster Food and Container Institute. These three lots are identified as C1, C2, and C3.

TABLE 2

Comparison of keeping time of lots of bacon of varying salt and moisture content

Lot No.	NaCl	Moisture	Moisture:salt ratio	Days required for 50 percent of cans to swell at 37° C.
	(percent)	*(percent)*		
1	1.79	22.66	12.7:1	58
1A	1.71	22.78	12.7:1	84
2	2.36	20.77	8.8:1	167
3	2.88	21.35	7.4:1	117
3A	2.99	22.81	7.6:1	..... *
4	2.62	21.45	8.2:1	..... *
5	3.03	21.84	7.1:1	..... *
5A	2.90	22.37	7.7:1	..... *
C1	1.45	23.35	16.0:1	50
C2	1.02	22.37	21.9:1	65
C3	1.26	20.28	16.0:1	70

* None swollen in 1 year.

It is noteworthy that Lots 1 and 1A, as well as the three commercial lots, all had a salt content of less than 2 percent, moisture:salt ratios as wide as or wider than 12.7:1 and relatively short keeping periods. The relatively long keeping time shown by Lots 3, 3A, 4, 5 and 5A may be due, in part, to the fact that all the bacon in this experiment was inoculated with pure cultures of suspected spoilage organisms which may not, in fact, have been as virulent as supposed. However, the results do indicate a positive advantage from heating in the smokehouse. In order to assure rapid spoilage for comparison of processing methods, later lots of bacon were inoculated with washings from cans of swollen bacon. The results of two such experiments are shown in Table 3.

TABLE 3

Comparison of two groups of heated and unheated bacon stored at 37° C.

Lot No.	Process	NaCl	Moisture:salt ratio	Storage behavior
		(percent)		
39	Smoked only	3.48	6.87:1	All cans swollen in 19 days
40	Smoked and 6-day heating	3.60	4.95:1	None swollen in 120 days
47	Smoked only	1.86	11.52:1	All cans swollen in 13 days
48	Smoked and 6-day heating	1.99	9.36:1	All cans swollen in 29 days

Lots 39 and 40 present a particularly interesting comparison. The bacon in these lots, as in all others used in this project, consisted of paired sides treated exactly the same, except for the 6-day heating period after smoke to which Lot 40 was subjected. The remarkable difference in keeping time clearly demonstrates the value of reducing the moisture:salt ratio to 5:1 or lower. Lots 47 and 48 show that 6 days' heating after smoking is not in itself effective in reducing spoilage when it does not result in sufficiently lowered moisture: salt ratios.

A comparison of two heated and two unheated lots of bacon is shown in Table 4. All bacon was processed according to the standard procedure described and Lots 32 and 44 were heated for 6 days after the normal smoking period. The Sorenson values tabulated show that proteolysis was retarded in the heated lots and a comparison of the free fatty acid values reveals that, although the heating treatment did increase the initial free fatty acid content slightly, the development of free

TABLE 4

Comparison of two groups of heated and unheated bacon

Days stored at 37° C.	Vacuum (inches Hg.)				Sorenson value (mg. amino N per gm. total N)				Free fatty acid (as percent oleic acid per gm. of fat)				Bacterial count (organisms per gm.)	
	Unheated		Heated		Unheated		Heated		Unheated		Heated		Unheated	Heated
	Lot 31	Lot 43	Lot 32	Lot 44	Lot 31	Lot 43	Lot 32	Lot 44	Lot 31	Lot 43	Lot 32	Lot 44	Lot 43	Lot 44
0	23	....	23	....	27	32	37	40	.95	1.50	1.04	2.03	.....	.....
12	18	*	21	20	50	49	41	45	2.30	3.61	1.57	2.93	5,100,000	12,300
26	17	12	20	20	65	51	51	45	4.26	4.38	2.17	3.99	154,000	15,900
40	20	13	22	19	71	63	48	48	6.58	5.45	3.10	4.38	13,400,000	5,500
54	13	12	19	19	87	74	48	56	8.31	6.68	3.71	5.11	450,000	0
68	12	14	18	19	93	79	56	60	10.07	7.51	4.29	7.00	348,000	4,200
82	13	10	18	18	101	87	57	60	11.31	8.55	5.21	6.58	460,000	26,000
96	0	10	18	20	117	88	65	61	12.09	9.59	6.00	7.94	110,000	1,000

* Leak in can.

acidity by lipolysis during storage was also greatly retarded. The bacterial counts, although erratic, are significantly lower in all cases, for the heated group. All lots were given the same initial inoculation.

Since the drying of bacon in a heated smokehouse depends on convection to bring dry heated air in contact with the sides of bacon, it was thought that lower moisture:salt ratios might be attained by drying the bacon in a cabinet dehydrator with the use of forced air circulation. Two lots of bacon were prepared by standard procedures and subdivided; half of each lot was analyzed immediately on removal from the smokehouse and the other half was held for 6 days in the drier at 120° F. (49° C.), with forced air circulation. The moisture:salt ratio in one case was reduced from 9.74:1 to 6.00:1 and in the second case from 8.70:1 to 7.49:1. Although these reductions were no greater than could be obtained by heating in the smokehouse for the same period with natural air circulation, a further comparison was made by drying two lots of paired sides by the two methods. Lot 37, which was dried by 6 days of heating in the smokehouse after smoking, showed a weight loss of 7.20 percent during the drying period and a final moisture:salt ratio of 5.32:1. Lot 38, which was dried in the cabinet drier for 6 days after smoking, showed a weight loss for the drying period of 7.22 percent and a final moisture:salt ratio of 5.24:1. From these results it was concluded that the advantages, if any, of drying by forced circulation of heated air were not great enough to make this method preferable to smokehouse drying.

It is well recognized in packinghouse practice that to avoid excessive weight losses bacon must not be stacked too high during curing. It is also well known that frozen meat, when thawed, shows a considerable "drip" or moisture loss. These two facts suggested that, if bacon were frozen, thawed, and pressed before curing, or pressed either during or after curing, a considerable quantity of moisture might be eliminated. However, a number of experiments designed to test this hypothesis failed to demonstrate any advantage in favor of this method of drying.

Isolation and identification of organisms from spoiled bacon. The objective of the bacteriological phase of this project was to identify the organisms responsible for spoilage of mildly-cured, sliced, vacuum-packed bacon. Although there are factors other than can swelling involved in the spoilage of this product, most of the bacteriological work was directed toward an understanding of the causes of swelling of the bacon cans when stored at a relatively high temperature.

Tubes of Jensen's medium were inoculated with bacon from a swollen can and incubated at 37°C. Dilutions were made from those tubes in which there was evidence of gas production and this material was streaked on veal infusion agar. The plates were incubated at 37° C. and isolated colonies were picked for identification. Approximately 80 percent of the more than 100 organisms isolated and studied were micrococci and 4 percent were staphylococci. All of these organisms produced acid in various carbohydrate media but none produced gas in Dunham fermentation tubes. Aerobic, spore-forming bacilli of the *B. subtilis* group have been described *(6)* as the causative agent of swelling in canned spiced ham and luncheon meats. However, strains of these organisms were isolated only twice from swollen cans of bacon.

The possibility that an anaerobic sporeformer might be involved in the spoilage of canned bacon was considered. Swollen cans of the product were heated for various periods of time in a water bath prior to opening to kill non-sporeformers. Isolations were made with pork heart medium for enrichment. Two strains of the *Cl. butyricum* group were isolated by this procedure. These organisms produced acid and gas from dextrose, lactose, sucrose, maltose and mannitol but not from xylose, did not form indol and gave slight H_2S production. Because of the heavy growth of micrococci originally present in the bacon, inoculation of canned bacon with these organisms did not yield proof of their ability to cause swelling of the cans. Furthermore, the fact that these *Clostridia* could not be isolated from every can of spoiled bacon indicated that there were other organisms involved.

Fifty-one strains of micrococci, which were previously isolated from swollen cans, were tested for their ability to produce gas in Jensen's medium. Of these, 36 strains produced gas in this medium, the majority being positive within 24 hours. It will be recalled that no strain of micrococcus isolated in this study produced demonstrable gas in Dunham fermentation tubes.

The production of carbon dioxide by bacon organisms was measured in fermentation tubes of the Eldridge type made by connecting two 50 ml. Erlenmeyer flasks with a glass tube. Ten ml. of medium were placed in one flask and the whole assembly sterilized. At the time of inoculation, the other flask was filled with 5 ml. of standard barium hydroxide solution. These fermentation tubes were incubated for 1 week at 37° C. and the CO_2 determined by titrating the barium hydroxide with standard acid. Two blanks (uninoculated tubes) were used in each run and the difference in titration value between the blanks and the inoculated flasks was used to calculate the amount of CO_2 produced by the organism in question.

The results with 51 strains of 7 species of micrococci and 2 species of sporeforming bacilli are summarized in Table 5. The micrococci reported were all organisms commonly found in bacon and are generally considered to be inert so far as keeping quality is concerned. However, there is little doubt that they were responsible for the swelling of the bacon cans. It is also interesting to note that all strains which failed to produce gas in Jensen's spiced ham medium did produce CO_2 which was measurable by the Eldridge technique. It seems

TABLE 5

Gas production by bacon organisms

Organism	Number of strains	Number producing gas in Jensen's medium	mg CO_2 produced under conditions of test		
			Average	Least	Most
M. perflavus	12	8	7.7	4.8	8.9
M. candidus	2	0	8.0	6.4	9.5
M. percitreus	5	4	5.8	0.8	9.1
M. nitrificans	11	9	3.6	0.2	22.0*
M. epidermidis	11	9	3.2	0.0	8.2
M. conglomeratus	9	6	2.6	1.2	6.7
M. species	1	0	8.7	....	....
B. megatherium	1	0	0.9	....	....
B. pumulis	1	0	0.0	....	....

* Based on single determination, not included in average.